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Appropriate methods of malaria control in acute and post-emergency settings

Thesis submitted to the University of London
for the degree of
Doctor of Philosophy
November 2005

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Abstract

This thesis examined appropriate tools for the control of malaria in complex emergencies, focusing on the refugee camp environment. Tools that may reduce the transmission of malaria, both through vector control as well as antimalarial drug treatment with regimens demonstrating gametocytocidal activity were evaluated. Field work took place in Pakistan.

Insecticide pre-treated plastic sheeting, canvas tents and blankets were examined in insectary bioassay and overnight outdoor platform tests to determine their potential for personal protection and "mass-effect" malaria vector control. These materials may be useful for use at the early stages of an emergency when an insecticide spray campaign or delivery of insecticide-treated nets may be hampered by logistic and organisational constraints and/or be inappropriate due to the shelters in use at this time.

Insecticide-treated plastic sheeting (ITPS) resulted in treatment induced anopheline mortalities between 17 and 43% in 5 of 7 overnight platform trials with wild host-seeking mosquitoes. Induced mortality in wild mosquito populations on insecticide-treated tents also fell within this range. A treatment induced 4-fold reduction in blood-feeding on tents was seen but an inconsistent impact on blood-feeding was seen for ITPS. A decline in insecticidal efficacy of pre-treated ITPS over a period of 13.5 months weathering was demonstrated, though persistence was considerably better than on sheeting sprayed with insecticide. Promising initial data of treatment induced mortality on insecticide-treated blankets were not replicable and more work is needed, perhaps with a revised testing protocol.

Insecticide-treated nets may be a feasible option during more sustained emergencies when procurement has been possible, people live in shelters suitable for erecting nets and the environment allows delivery of nets with appropriate health education. Long-lasting insecticidal nets (LLINs) are particularly suitable for a transient population in which retreatment of conventionally treated nets is problematic, for example in internally displaced people (IDPs) or refugees who may return home. Two candidate deltamethrin LLINs were compared to conventionally treated nets in insectary bioassay, overnight platform assays and high pressure liquid chromatography assays. PermaNet™ 2.0 was shown to have superior performance by all test criteria than conventionally treated nets. Dawa™ net was not superior to conventionally treated nets.

Two randomised controlled trials of antimalarial drug treatment regimens were carried out; in falciparum patients and in vivax patients. The study against falciparum malaria was a six arm study including the current first and second line treatments in the region, chloroquine (CQ) and sulphadoxine-pyrimethamine (SP), as well as combinations of each of these drugs with primaquine or artesunate (AS). Chloroquine resistance was high; CQ monotherapy achieved only 23% clinical cure. CQ+AS gave an improved cure rate (72% clinical cure) over CQ alone ($P<0.001$). Some resistance to SP was seen, with 10% clinical failure. SP+AS achieved 100% clinical cure. The odds of a patient carrying gametocytes on or after day 7 is associated with the presence of gametocytes on day 0, regardless of treatment given. Both primaquine and artesunate treatment regimens reduced gametocyte persistence compared to the monotherapies. This was most marked for artesunate and in patients without patent gametocytaemia on day 0.

The study against vivax malaria compared the standard CQ regimen with a combination of SP+AS, promoted as the 1st line regimen for falciparum malaria in the region. With approximately 80% of the malaria in the region caused by the vivax parasite the likelihood of misdiagnosis or incorrect treatment of vivax cases as falciparum malaria is high. This would also be the case in many post-emergency settings, where both parasites occur, where the quality of the health systems are unlikely to ensure a good level of accurate differential diagnosis. Where the falciparum first line treatment remains chloroquine this issue is irrelevant, however, with SP+AS concerns over the efficacy of a combination including SP, thought to have relatively poor activity against vivax, needed to be addressed. Chloroquine treatment is known to clear vivax gametocytes rapidly, but it is not clear if SP+AS would have the same effect.

Both CQ and SP+AS had high efficacy for vivax malaria with SP+AS performing better over an extended period of follow-up (42 days) than CQ. No difference was seen in gametocyte carriage after treatment in the 0-28 day period, though patients with reappearance of trophozoites in the 28-42 day period were also carrying gametocytes. Fewer patients had reappearance of any parasites in the SP+AS group than in the CQ group.

The results of these studies are discussed alongside other work in this field and recommendations for suitable malaria control tools for emergencies are given. Future research needs are highlighted.

Dedicated to JoJo

Acknowledgements

I would like to thank the following:

Mark Rowland, my PhD supervisor, for his enthusiastic and ready support of my PhD and other work. His advice and guidance were unstinting, without them this work would not have begun, progressed or been completed.

Other staff and students at LSHTM who have provided advice and assistance: Jo Lines, Chris Curtis, Clive Davies, Mohammad Kayedi, Diarmud Campbell-Lendrum, Jon Cox, Alison Yates, Chris Whitty, Jayne Webster, Cheryl Whitehorn, Ilona Cainero, Patricia Aiyenuro, Sylvia Meek and Jan Kolaczinski.

HealthNet International's Refugee Programme and Peshawar office for their technical, logistic and personal support, and for allowing much of the research to take place at their field sites. In particular: Nasir Mohammed, Mushtaq Ahmad, Mohammed Kamal, Hameed Rehman, Irfanullah Khan, Abdullallah, Hannif, Gul Nabi, Safia, Mathiuallah, Raof, Toby Leslie, Sayed Nazir, Richard Reithinger, Panna Erasmus and Jan Kolaczinski.

The Afghan refugees of Azakhel camp who assisted with the entomological night work, and the refugees of Adizai, Yakka Ghund and Mohammad Khoja camps who agreed to participate in the drug trials.

Vestergaard-Frandsen, SiamDutch Mosquito Netting, TDR and WHOPES for providing the funding for the field research.

Ole Skovmand for his helpful suggestions and comments.

The friends made during my time in Pakistan and Afghanistan: Toby Leslie, Richard Reithinger, Annick Lenglet, Sara Petersson, John Dempsey, Peter MacHolst, Jamie Terzi, Brian White and Dan Ryan.

My brother, Chris, for making a PhD look so much fun. If only I had believed him about how long it takes to write-up.

My parents, Dick and Marcia, for their emotional and financial support and for organising the most wonderful wedding whilst I was caught up in work and thesis writing.

My husband, Jan Kolaczinski, for his patience, advice, encouragement, support, plant watering and cooking.

Lucy and Jack for not being too annoying, most of the time.

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Chapter 1. Literature Review and Introduction

1.1 Healthcare in complex emergencies

The difficulties associated with providing preventative and curative health services to displaced populations are not new. Conflicts and natural disasters have resulted in populations fleeing and re-settling throughout human history. The recent development of the term “complex emergency” or “complex political emergency” reflects an increased number, and increase in the awareness, of emergencies resulting from a combination of causes: political, economic, social, ethnic, religious and environmental (Burkle, 1999; Schull & Shanks, 2001).

The United Nations High Commissioner for Refugees (UNHCR) estimated that at least 17 million people were directly affected by complex emergencies in 2004. These were mainly refugees (9.7 million) and internally displaced people (IDPs, 4.4 million). Estimates of affected populations vary; the Representative of the UN Secretary-General on Internally Displaced Persons puts the number of IDPs worldwide at 20-25 million. In addition to these figures, affected groups outside the UNHCR mandate, such as refugees or IDPs who have returned home should be included as populations affected by complex emergencies (UNHCR, 2004).

Whilst each complex emergency is unique, as a result of the different combinations of causes and influences in each case, there are similarities in how these situations arise and evolve. Though unrest may have been simmering for some time, international attention is usually first attracted when violent conflict erupts. This violence often leads directly to mass population displacements; the concomitant political and economic upheaval leads to a breakdown of institutional support mechanisms including health services (Hansch & Burkholder, 1996; Biberson, 1999). This initial period may see very high mortality levels (an “acute” emergency) and this period may be either rapidly resolved or lengthy. At some point, as steps to deal with the emergency are taken, some stabilisation will occur and mortality rates should fall. The subsequent “post-emergency” stage may, in rare fortuitous cases, lead directly into conflict resolution and a process of country re-building, as in the example of East Timor (Kolaczinski & Webster, 2002). However, more commonly, the emergency moves into a “chronic” stage where conflict is on-going, although possibly intermittent, state rebuilding is unlikely, and the possibility of rebuilding centralised health services is therefore also remote. There may be some fluctuation between the acute and chronic stages as sporadic conflict erupts: pockets of

populations living in acute stage and post-emergency environments may be scattered around the one country, for example the situation until recently in Afghanistan (Rowland, 2001) and the current situation in Somalia.

For health care delivery each of these stages presents different problems and different opportunities (Waldman & Martone, 2002; Kolaczinski & Webster, 2003). Box 1-1 shows some generalised characteristics of the different stages that may occur during an emergency.

Box 1-1. Generalised characteristics of the main phases of a complex emergency which have implications for the delivery of health care

Acute phase

- Crude mortality > 1 death per 10,000 per day
- Flimsy and temporary shelters with high concentrations of people
- Attention focused on emergency healthcare, delivery of food, blankets and shelter materials
- Inadequate health care facilities and high patient case loads
- Unpredictable security
- Limited inter-agency co-ordination
- Relatively large amounts of short-term funding (6-12 months)
- Short-term vision of implementation
- Health care almost exclusively provided by non-governmental organisations (NGOs)
- Strong presence of NGOs with expertise in emergency situations

Chronic phase

- Crude mortality < 1 death per 10,000 per day
- Some areas of a country may remain in the acute phase, others move towards post-emergency
- More stable and permanent housing structures
- Improved security
- Improved inter-agency co-ordination
- Improved healthcare provision
- A newly established administration or government with often only focal areas of control
- Health infrastructure not yet rebuilt
- Lack of trained healthcare staff
- Much of health care still provided by NGOs
- Increasing presence of NGOs with development expertise
- Persisting presence of agencies with a focus and skill base suited for emergency interventions

[Modified from: Kolaczinski & Graham, 2004]

An acute emergency is defined as a situation where the crude mortality rate is greater than one death per 10,000 people per day (Spiegel *et al.*, 2002). This abnormal elevation of morbidity and mortality is most often the result of poor sanitation, lack of clean water, crowded and unsatisfactory living conditions, and lack of sufficient basic health services (Toole and Waldman, 1990). Mass population displacement often leads to the establishment of camps.

A proportion of the population affected by a complex emergency will live through it in a non-camp environment, and the issue of providing health care to this sector of the population is also problematic. However, the agencies involved in health care delivery at the acute stage of an emergency are often working in a camp environment. In such an environment strategies shown to be effective in stable situations may need to be adapted and closely monitored for their effectiveness or may not be logistically feasible at this chaotic early stage. Numerous guidelines for health care implementation in such environments exist (*e.g.* Simmonds *et al.*, 1983; Mears & Chowdry, 1994; MSF, 1997; UNHCR, 1999).

A complex emergency is considered to have moved into the post-emergency phase once the crude mortality rate has fallen to less than 1 death per 10,000 per day (Speigel *et al.*, 2002). Although an arbitrary delineation there are general characteristics which will have led to this decline in the death toll: improvements in security, agency co-ordination, healthcare and provision of basic necessities such as clean water and shelter. The population of a camp environment, if permitted, will quickly begin to construct more stable and permanent shelters than the tents and sheeting used in the first few weeks (Graham, 2004). A more secure, stable and better co-ordinated camp environment has, by this stage, been established. These characteristics of a post-emergency environment allow different possibilities for health care. As an emergency is protracted the priorities and challenges will change further and increasingly resemble those of stable settings, though of course the challenges will be compounded by some unique characteristics of the post-conflict environment (Box 1-1).

Given the fluid state of a complex emergency setting it is important that this changing environment is taken into account when planning health care activities to ensure that suitable strategies are adopted to meet the evolving needs of the population.

1.2 Malaria and its control in complex emergencies

When a complex emergency occurs in a malaria endemic area malaria incidence is likely to rise and epidemics are a threat (Rowland & Nosten, 2001). This applies both to populations living in refugee or IDP camps as well as those living in a non-camp environment. There are several reasons for this:

- The health system and existing control programmes are likely to have collapsed (Kalipeni & Oppong, 1998)
- The health systems and existing control programmes in neighbouring countries may be overwhelmed by refugee influx, e.g. Pakistan after the influx of Afghan refugees following the Soviet invasion (Rowland *et al.*, 2002b)
- Non-immune individuals may have been displaced into malaria endemic areas (Thomson, 1995; WHO, 2000; Martens & Hall, 2000; Rowland, 2001; Worrall *et al.*, 2004).
- Inadequate health facilities, poor security and high patient caseloads combine to make treatment access limited (Thomson, 1995; WHO, 2000; Martens & Hall, 2000; Rowland, 2001; Worrall *et al.*, 2004).
- In camps, cramped living conditions provide increased man-vector contact promoting increased transmission of the malaria parasite (Thomson, 1995; WHO, 2000; Martens & Hall, 2000; Rowland, 2001; Worrall *et al.*, 2004).
- Governments in regions undergoing complex emergencies may be unwilling or unable to fulfil their obligations or may be selective as to which population subgroups are to be targeted for assistance (Weiss & Collins, 2000).

The challenges facing healthcare delivery in this environment mean that techniques and implementation strategies for the control of malaria must often be different from those used in stable settings. Possible strategies for malaria control¹ are discussed below with a review of the relevant literature; their suitability for use in emergency settings is discussed.

¹Throughout this thesis malaria "control" is considered to embrace control of morbidity and mortality levels both by reduction of transmission and by the effective management of cases. Malaria "prevention" refers purely to the reduction of transmission.

1.3 Vector control interventions for the control of malaria in complex emergencies

1.3.1 Insecticide-treated nets

Insecticide-treated nets (ITNs) are highly efficacious in reducing mortality and morbidity from malaria (in particular in children, on whom most studies have focused). A recent Cochrane review (Lengeler, 2004) compiled data from fourteen trials comparing malaria morbidity and mortality in children sleeping under ITNs compared to untreated nets or no nets. ITNs reduced the number of uncomplicated *Plasmodium falciparum* malaria episodes in areas of unstable malaria by 50% compared to no nets and by 39% compared to untreated nets; and in areas of stable malaria by 62% compared to no nets and 43% compared to untreated nets. Positive impacts on child mortality, severe malaria, parasite prevalence, high parasitaemia, splenomegaly and haemoglobin levels were also demonstrated (Lengeler, 2004).

ITNs have become the favoured vector control tool for malaria in many settings; ranging from humanitarian emergency relief to use as a component of national malaria control programmes in stable situations. However, evidence for the effectiveness of ITNs, *i.e.* their impact on malaria morbidity and mortality in everyday use (rather than trial conditions) under different field conditions is limited. Where it has been studied, for example in case-control studies in well established refugee settlements in North West Pakistan and in Eastern Afghanistan, the odds of ITN users being infected with malaria was significantly lower than for non-users, with odds ratios of 0.22 (95%CI 0.09-0.55) in NWFP (Rowland *et al.*, 1997a) and 0.41 (95% CI 0.25-0.66) in E. Afghanistan (Rowland *et al.*, 2002a).

Whether ITNs are a suitable tool for implementation during a complex emergency is determined by various factors. Box 1-2 outlines some of the considerations to take into account for the use of ITNs as a malaria control method. The implications these considerations will have on the appropriateness of ITNs in an emergency setting are described.

Box 1-2. Some operational considerations for deployment of ITNs

Consideration	Implications in an emergency setting
<ul style="list-style-type: none"> Requires health education and possibly behaviour change if populations are not used to using nets, to include: <ul style="list-style-type: none"> how best to use the nets to ensure maximum protection an understanding of the health benefits so that these items are not sold on the need for re-treatment if conventional nets are used. 	<ul style="list-style-type: none"> Design, pre-testing and implementation of a successful health education campaign is unlikely to be possible during the early phase of an acute emergency, the skills may not be available, security may not allow it and it would take time. Feasible in some post-emergency settings or in chronic emergencies.
<ul style="list-style-type: none"> Conventionally treated nets need to be re-treated at least once per year, preferably every 6 months. Health education is required to create an awareness of the need for re-treatment. Some expertise is required to ensure safe handling of insecticides and correct impregnation. Only free mass re-treatment campaigns have achieved high coverage of re-treatment, these are logistically and financially expensive. WHO endorsed long-lasting insecticide-treated nets (LLIN) do not have this disadvantage. 	<ul style="list-style-type: none"> A net re-treatment campaign may be feasible 6 months into an emergency provided the skills and equipment are in place and security allows. In the post-emergency phase refugees or IDPs may begin to return home, retreatment of any conventionally treated ITNs may not be possible after this. LLIN would be particularly suitable for use in a complex emergency.
<ul style="list-style-type: none"> Nets are “flexible”, <i>i.e.</i> can be moved with the owner. 	<ul style="list-style-type: none"> Useful to provide on-going protection to refugees or IDPs when they begin to return home. Can easily be sold on if the health benefits are not fully appreciated, surely a temptation for very poor populations such as refugees
<ul style="list-style-type: none"> Need to be erected over a sleeping place. 	<ul style="list-style-type: none"> For a population living under temporary shelters such as plastic sheeting or tents there may not be enough room or possibilities for support to make erection of the net possible. Once more permanent structures are erected this may be less of a problem.
<ul style="list-style-type: none"> Net and insecticide need to be ordered and delivered, which may take several months. Alternatively they need to be stock-piled. 	<ul style="list-style-type: none"> Often therefore impossible to provide nets in the very early stages of an emergency.

[Modified and expanded from: Kolaczinski & Graham, 2004]

In particular, the need to procure and import the nets and the problems of erecting nets in small and flimsy shelters make it doubtful that ITNs are a suitable tool for malaria prevention during the acute phase of an emergency in most cases (Rowland *et al.*, 2004). In the more established environment of a chronic emergency setting or in the more stable environment of a post-emergency setting they may be a more appropriate or feasible choice: procurement of nets should have been possible by this stage and with the initial high mortality rate under control it is realistic to concentrate on establishing appropriate distribution mechanisms and the development and delivery of health education messages. Successful subsidised sale of ITNs in well-established refugee camps in Pakistan and in the chronic emergency of Afghanistan has been possible (Kolaczinski *et al.*, 2005) with the effectiveness of this approach proven (Rowland *et al.*, 1997a; 2002).

One of the distinct advantages of ITNs as a prevention tool during the post-emergency period is that they can be used to provide on-going protection to returning refugees or IDPs. However, the disadvantage of conventionally treated nets is that they require re-treatment, limiting the period of protection provided to the returnees if no retreatment services are on offer in the area to which they return.

Persistence of insecticide on nets

Several studies examining the persistence of insecticides on ITNs in the late 1980s, 1990s and early 2000s focused on washing, the main factor known to strip insecticide from nets. As interest in ITNs and recognition of the problems of retreatment grew, there was a surge of interest in the relative persistence of the various insecticides and formulations available. Table 1-1 summarises several studies which have looked at the effect of washing on ITNs. Indicators used have been either direct analysis of the insecticide deposit or performance monitoring through contact bioassay or overnight studies in experimental huts.

The most comprehensive studies, and those including analysis of insecticide deposits, were carried out in the early 1990s when some of the insecticide formulations currently available (*e.g.* deltamethrin SC) were not yet in use. Most frequently just a few washes resulted in extremely low insecticide deposits. Permethrin EC (40:60) on polyester nets treated conventionally or in a hot acid bath were the most persistent when insecticide deposits were examined (Lindsay *et al.*, 1991).

The effect that this rapid stripping of the insecticide deposit by washing has on the performance of the net is less clear. In the two studies where both of these indicators are presented it is apparent that whilst much of the insecticide can be stripped from the net, the mosquito mortality seen in bioassay may be only slightly reduced (Miller *et al.*, 1991; Lindsay *et al.*, 1991).

In the later studies, which only examined performance of the nets in inducing mortality, the best persistence was seen on a combination of net fabrics were treated with permethrin mixed with polyethylene (Miller *et al.*, 1995) and on polyester nets treated with either lambdacyhalothin (EC and CS) or deltamethrin SC (Curtis *et al.*, 1996).

Despite variations in scale between trials it is clear that insecticide is stripped from nets of all fabrics by washing, with a resulting decline in net performance. With such conventional insecticide treatments the re-treatment of nets will remain a repeated need throughout the physical life of the net.

Table 1-1. Published studies examining the effect of washing on ITNs conventionally treated¹ with various insecticides and formulations on different fabrics

Study	Insecticide	Formulation ²	Dosage mg ai/m ²	Results for washed nets compared to unwashed nets	Material	Outcome measure ³	Washing protocol
Rozendaal <i>et al.</i> , 1989	Permethrin	EC	2000	24h mortality: reduced by 50% after 2 washes	Cotton	Mean % 24h mosquito mortality following 2 min contact bioassays using <i>An. darlingi</i>	Machine wash in cold water Washed twice
			500	24h mortality: reduced by 21% after 2 washes			
			250	24h mortality: reduced by 91% after 2 washes			
Miller <i>et al.</i> , 1991	Cypermethrin	EC	100	Insecticide deposit: 84% lost after 3 washes 24h mortality: reduced by ~7% after 3 washes	Nylon	1) Insecticide deposit (mg ai /m ²): measured by gas chromatography 2) Mean % 24h mortality of <i>An. gambiae</i> mortality in experimental huts	Local methods and products Washed 3 times at start of trial
	Deltamethrin		25	Insecticide deposit: 100% lost after 3 washes 24h mortality: reduced by ~13% after 3 washes			
	Lambdacyhalothrin		25	Insecticide deposit: 85% lost after 3 washes 24h mortality: reduced by ~12% after 3 washes			
	Permethrin		500	Insecticide deposit: 100% lost after 3 washes 24h mortality: reduced by ~10% after 3 washes			
	Pirimiphos-methyl		1000	Insecticide deposit: 100% lost after 3 washes 24h mortality: reduced by ~15% after 3 washes			

¹ i.e. not treated by the manufacturer at factory level

² EC=emulsifiable concentrate; SC=suspension concentrate; CS= capsule suspension; WP = wettable powder; WG= wettable granules

³ Test methods only described for results included in the table

Study	Insecticide	Formulation	Dosage mg ai/m ²	Results for washed nets compared to unwashed nets	Material	Outcome measure	Washing protocol
Lindsay et al., 1991	Cyfluthrin	EC	100	Insecticide deposit: 82% lost after 2 washes 24h mortality: reduced by ~10% after 4 washes	Polyester	1) Mean insecticide deposit (mg ai/m ²) measured by gas chromatography 2) Mean % 24h mosquito mortality following 30 second exposure bioassays with <i>An. gambiae</i>	Local washing methods and products 4 washes at 2-weekly intervals
			200	Insecticide deposit: 90% lost after 2 washes			
	Cyphenothrin	EC	100	Little to no initial uptake of insecticide by the net			
			200				
	Cypermethrin	EC	100	Insecticide deposit: 75% lost after 2 washes 24h mortality: remained 100% after 4 washes			
			200	Insecticide deposit: 67% lost after 2 washes			
	Deltamethrin	EC	25	Insecticide deposit: 90% lost after 2 washes 24h mortality: reduced by ~35% after 2 washes			
			50	Insecticide deposit: 83% lost after 2 washes			
	Pheothrin	EC	100	Little to no initial uptake of insecticide by the net			
			200				
	Fenpropathrin	EC	100	Insecticide deposit: 80% lost after 1 wash			
			200	Insecticide deposit: 88% lost after 2 washes			
	Lambdacyhalothrin	EC	25	Insecticide deposit: 66% lost after 2 washes 24h mortality: no change after 4 washes (100%)			
			50	Insecticide deposit: 46% lost after 2 washes			
	Permethrin (40:60)	EC	200	Insecticide deposit: 63% lost after 2 washes			
			500	Insecticide deposit: 37% lost after 2 washes			
	Permethrin (25:75)	EC	200	Insecticide deposit: 47% lost after 2 washes 24h mortality: reduced by ~56% after 2 washes			
			500	Insecticide deposit: 62% lost after 2 washes			
	Permethrin (25:75 at 97°C and pH 3.4)	EC	200	Insecticide deposit: 39% lost after 2 washes			
			500	Insecticide deposit: 37% lost after 2 washes			
	Permethrin (25:75 with 100 mg ai/m ² of 'Agris 90')	EC	200	Insecticide deposit: 73% lost after 2 washes; 24h mortality: remained > 95% after 4 washes			
			500	Insecticide deposit: 63% lost after 2 washes			

Study	Insecticide	Formulation	Dosage mg ai/m ²	Results for washed nets compared to unwashed nets	Material	Outcome measure	Washing protocol
Jana-Kara <i>et al.</i> , 1994	Deltamethrin	Flowable	6 doses ranging from 5 – 25 mg ai/m ²	24h mortality: reduced by ~48% after 1 wash	Cotton	Mean % 24h mosquito mortality following 30 s, 1 min and 3 min exposure bioassays using <i>An. stephensi</i> Mortalities presented as means of all the exposure periods and doses	1 hand wash with local method and products
				24h mortality: reduced by ~73% after 1 wash	Nylon		
				24h mortality: reduced by ~59% after 1 wash	Polyethylene		
		WP		24h mortality: reduced by ~73% after 1 wash	Cotton		
				24h mortality: reduced by ~70% after 1 wash	Nylon		
				24h mortality: reduced by ~49% after 1 wash	Polyethylene		
	Lambdacyhalothrin	EC		24h mortality: reduced by ~78% after 1 wash	Cotton		
				24h mortality: reduced by ~70% after 1 wash	Nylon		
				24h mortality: reduced by ~76% after 1 wash	Polyethylene		
Miller <i>et al.</i> , 1995	Permethrin	EC	500	24h mortality: reduced by ~90% after 3 washes	Synthetic / Cotton / Mixture	Mean % 24h mortality following 3min exposure bioassays All fabrics tested, overall means	Local methods and products No. of washes reported by net owner
	Permethrin	EC with polystyrene	500	24h mortality: reduced by ~22% after 3 washes			
	Lambdacyhalothrin	EC	25	24h mortality: reduced by ~53% after 3 washes			
Curtis <i>et al.</i> , 1996	Lambdacyhalothrin	EC	10	24h mortality: remained > 95% after 1 wash and 2 further months use (unwashed = 8m use)	Polyester	Mean % 24h mosquito mortality in 3min exposure bioassays with <i>An. gambiae</i>	1 hand wash with local method and products
			5	24h mortality: reduced by ~47% after 1 wash and 3 further months use (unwashed = 7m use)			
		CS	10	24h mortality: remained > 95% after 1 wash and 2 further months use (unwashed = 8mo use)			
	Deltamethin	SC	25	24h mortality: reduced by ~28% after 1 wash and 3 further months use (unwashed = 7m use)			
	Etofenprox	EC	200	24h mortality: reduced by ~85% after 1 wash and 3 further months use (unwashed = 12m use)			

Study	Insecticide	Formulation	Dosage mg ai/m ²	Results for washed nets compared to unwashed nets	Material	Outcome measure	Washing protocol
Jawara <i>et al.</i> , 1998	Alphacypermethrin	SC	40	24h mortality: reduced by 81% after 2 washes	Nylon / Cotton / Other	Mean 24 h mortality following 3 min exposure bioassays with <i>An. gambiae</i> on nets after 3 months field use	Local methods and products No. of washes reported by net owners
	Permethrin	EC	500	24h mortality: reduced by 100% after 2 washes			
	Lambdacyhalorthin	EC	10	24h mortality: reduced by 100% after 2 washes		All fabrics tested, overall means given	
Chirebvu & Nzira, 2000	Alphacypermethrin	SC 6%	30	MTKD: 84% longer on nets washed once	Polyester	Mean MTKD (min) of <i>An. arabiensis</i>	1 supervised hand wash by net owners after 3 months use (nets in everyday use)
		Dry 15%	30	MTKD: 11% longer on nets washed once	Polyester		
Asidi <i>et al.</i> , 2004	Carbosulfan	CS	200	No. dead: 65% lower on washed nets	Polyester	Mean no. of mosquitoes dead 24 h after exposure in experimental huts	5 hand washes over a 5 day period using local methods and products
	Alphacypermethrin	WG	20	No. dead: 15% fewer on washed nets (n.s) ¹			
	Lambdacyhalorthin	CS	18	No. dead: 13% more on washed nets (n.s)			
	Permethrin	EC	500	No. dead: 25% fewer on washed nets (n.s)			

¹ n.s = not significant. In this study significance values were given to the comparison of unwashed nets to nets washed 5 times, where the reduction in performance was not significant this is indicated. In none of the other studies presented in the table were significance values presented

Wash resistant insecticide-treatments

The search for a “long-lasting” insecticide treatment is not new. The different formulations of insecticides were (and are) developed with a view both to ease of use and application as well as the persistence on a specific surface (mud walls as opposed to fabrics for example); the studies outlined in Table 1-1 aimed to identify the longest persisting combination of insecticide, formulation and fabric. The idea of modifying the conditions of treatment was examined in two of the studies. Impregnation at high temperatures in an acid bath was shown to result in greatly increased persistence of permethrin EC at 500mg ai/m² on polyester fabric (Lindsay *et al.*, 1991), although field application of such a technique is unlikely. Miller and colleagues (1995) demonstrated that the addition of polystyrene to permethrin during impregnation also resulted in better persistence of the treatment.

In the mid-1990s a factory pre-treated net with enhanced persistence of insecticide after washing was developed by a Japanese company, Sumitomo. Olyset® is a polyethylene net treated with permethrin (40:60); the insecticide is incorporated into the polyethylene polymer prior to yarn extrusion (WHO, 2001a). Recently the World Health Organisation (WHO) has stimulated more industry players to develop treatment processes whereby the insecticide is more stably bound within or onto the fibres of the net, resulting in nets of an extended insecticidal life, these have gradually become known as ‘LLINs’: long lasting insecticide-treated nets. The current emphasis is on identification of a treatment that will resist washing and ensure the net remains efficacious for the physical life span of the net, usually described as 4 to 5 years¹. Olyset was evaluated by the WHO pesticide evaluation scheme (WHOPES) in 2001 and was given WHO “recommendation” as a LLIN, having been shown to be efficacious over an extended period (N’Guessan, 2001). In the early 2000s limited production capacity and narrow specification options meant Olyset was not widely used by governments or other agencies involved in malaria control.

PermaNet™ is a prototype ‘LLIN’ developed by the Danish textile company Vestergaard Frandsen. The initial tests of PermaNet gave contradictory results. Some showed PermaNet declining in efficacy (mosquito mortality after 3 minute exposure contact bioassays) after washing (Mosqueira *et al.*, unpub., Müller *et al.*,

¹The physical life-span of a net depends on the fabric (e.g. polyethylene is more durable than polyester) as well as the conditions of use (e.g. is the net used all year round or only seasonally?) the number of washes, and the presence of other factors that may result in rapid deterioration of the net condition (e.g. rodents chewing the fabric). Estimates given for a generalised life-span of a net vary considerably, and in most sub-Saharan settings where nets are used throughout the year it is unlikely that polyester nets would last much longer than 3 years.

2002). Other studies, also using close contact bioassay, showed PermaNet to have a good wash resistance Gonzales *et al.* (2002) and Kilian *et al.* (unpub.). When tested in experimental hut trials PermaNet has been shown to perform as well after 20 washes as unwashed (Mosqueira *et al.*, unpublished).

The methodology used in these initial studies was inconsistent and often inadequate. Only the Gonzales *et al.* (2002) and the Kilian *et al.* (unpublished) trials wash conventionally treated nets alongside the PermaNets for comparison. The variation in wash resistance of conventional net treatments shown in Table 1-1 illustrates that direct comparisons of conventional net treatments on the same type of fabric with the same insecticide are important, rather than resting on an assumption that persistence of insecticide for more than a few washes makes the net superior to a conventionally treated one.

1.3.2 *Indoor residual spraying*

The development of DDT in the early 1940s led to the first large scale malaria control programmes based on the indoor residual spraying of houses. Since that date many national programmes have relied on indoor residual spraying (IRS) as the primary method of malaria control. IRS has been shown to prevent morbidity and economic losses (de Zulueta *et al.*, 1980; Mills 1991; Rowland *et al.*, 1994; 1997).

Good malaria control has been demonstrated where campaigns are properly targeted and managed, such as the 51% protective efficacy demonstrated in well-established refugee camps by Rowland (1999). The only other efficacy data available from a refugee setting are also for well-established refugee camps where inhabitants lived in houses; in refugee camps in Eastern Sudan a reduction in mortality in camps sprayed with malathion was observed compared to unsprayed control camps, though no reduction was seen in clinical cases of malaria (Charlwood *et al.*, 2001).

However, efficacy does appears to depend on a spray campaigns being well organised (Rozendaal, 1997). Logistical and organisational problems have been blamed for the resurgence of malaria in India in the 1970s (Sharma & Mehrota, 1986) and the failure of some spray rounds in the refugee camps in Pakistan (Rowland *et al.*, 2004).

In the acute stages of an emergency, constraints similar to those impeding implementation of an ITN campaign are faced: trained spray-men, specialised

equipment, large quantities of insecticide, and good co-ordination between agencies are all required but generally absent at this early stage. Once the situation moves into a post-emergency or chronic emergency phase it may be more feasible to conduct a successful spray campaign.

The need for re-application of insecticide used in IRS compounds these difficulties, as with the need for re-treatment of ITNs. Only campaign style reapplication will suffice, meaning that the financial and logistic resources needed at the outset will have to be mobilised repeatedly. Although DDT persists for 6-12 months on wood and thatch this insecticide is now used only in areas where malaria vectors are resistant to other less toxic insecticides such as the pyrethroids. Organophosphates and pyrethroids are now most commonly used for IRS and these require reapplication after 3 – 6 months, with the upper estimate applying only to deltamethrin (Rozendaal, 1997).

The estimates for the duration of the effectiveness of insecticides depend on the use of a wettable powder which is more persistent on absorbent surfaces than emulsifiable concentrate or suspension concentrate formulations and is therefore considered the most durable formulation for IRS on wood and thatch walls. The persistence of an insecticide sprayed onto surfaces is dependant both on the formulation of the insecticide and on the type of surface. For example most insecticides are known to last longer on wood and thatch than on mud, which absorbs some of the insecticide and may also break it down chemically (Rozendaal, 1997). In the early stages of an emergency people are likely to be living in canvas tents or plastic sheeting, whichever is distributed for shelter. The persistence of insecticides on plastics and tent fabrics is discussed further below (section 1.3.3).

1.3.3 Alternative vector control interventions for an acute emergency

The main issues limiting the applicability of ITNs and IRS at the early stages of an emergency are: (i) the urgency to get a vector control intervention in place as soon as possible; (ii) the type of shelter in use which may be inappropriate for erecting a net or spraying with insecticide; (iii) the environment of limited co-ordination, poor security and in-demand logistical resources which hinders campaign style approaches, rigorous supervision and sensitisation and health education programmes. Interventions that would be more appropriate at this stage would therefore take these issues into account.

The urgency to get a vector control tool in place as soon as possible at this stage of heightened mortality suggests that the most appropriate interventions would be

those linked to the accepted priorities of emergency situations. There are now defined priorities to minimise mortality at the outset of an emergency, these are measles immunization and the provision of clean water, sanitation, food, shelter and emergency health care (Rigal, 1992; MSF, 1997; Schull & Shanks, 2001).

It has been suggested that shelter materials or blankets that are pre-treated with insecticide may be useful vector control tools at this stage. In 2000 Roll Back Malaria made a call for manufacturers to move into the development of such products (RBM, 2000). Shelter material and blankets are delivered as a priority as standard, no additional resources or organisation would be required to deliver insecticide-treated versions. The literature outlining the background and potential of these tools is reviewed below.

Insecticide-treated shelters

Treating of tents with insecticide was originally attempted as a method of malaria control suitable for nomadic peoples (Motabar *et al.*, 1974). This early work with DDT, dieldrin and hexachlorocyclohexane had little success due to the short persistence of the available formulations of these insecticides on fabrics (Motabar, 1974). The longer residual efficacy and suitability for textile treatment of the available formulations of pyrethroid insecticides has meant that successful treatment of temporary shelters such as tents has been possible in recent years. Military research on personal protection methods in the early 1990s made use of these new insecticides; methods to treat pitched tents with permethrin were developed (Qureshi, *et al.*, 1990). Deltamethrin-treated cloth, wrapped around the poles of a roofed shelter has been used (Xavier & Lima, 1986), when woven polypropylene was used as the material, rather than cloth, a good resistance to wash-off of the insecticide from rain was reported (G. White, Pers Comm., cited in Rozendaal, 1997).

Entomological evaluations have demonstrated a reduction in mosquito blood-feeding and an increase in knockdown and mortality in the presence of permethrin-treated tents. Permethrin-treated tents reduced *Aedes spp.* biting by over 80% (Schreck, 1991; Heal *et al.*, 1995) and achieved a mean 58% knockdown over a one year period of constant weathering (Schreck, 1991). Permethrin and deltamethrin-treated tents reduced *Anopheles stephensi* biting by about 40% and resulted in 75% mortality amongst blood-fed mosquitoes (Hewitt *et al.*, 1995).

Spraying of permethrin on tents has been used to control epidemic malaria in a population of nomadic Afghan refugees in Pakistan (Bouma *et al.*, 1996a) and has

been adopted as an operational possibility in similar situations. It is recommended in various handbooks for disease control in refugee camps (Thomson, 1995; MSF, 1997; UNHCR, 2000) and has been implemented by UNICEF in camps following widespread flooding in Mozambique. The disadvantage of such large scale activities in an acute emergency have been discussed above. There are other important issues limiting the usefulness of campaigns to spray refugee shelters:

- Permethrin sprayed on canvas tents of the 'single-fly' type (such as those used in acute stage emergencies) showed decay of residue within a few months of spraying the inner surfaces (Bouma *et al.*, 1996b).
- Plastic sheeting is often favoured over canvas tents as a shelter material for refugee camps, being cheaper to make, cheaper to airfreight and easier to stockpile (Rowland & Nosten, 2001). There have been concerns that plastic sheeting is unsuitable for spraying with insecticide as the insecticide formulations generally used may not adhere to the plastic (Meek *et al.*, 2000).

In early 2000 Roll Back Malaria and the Technical Support Network for RBM in complex emergencies began discussions with industry on the subject of pre-treated shelters, with the rationale that canvas tents and plastic sheeting pre-treated with insecticide would side-step the need for a spray campaign. It was also hoped that methods of insecticide treatment could be used that would result in a longer residual life than spraying provides. An early prototype of pre-treated sheeting weathered in a temperate climate did show good persistence of insecticide (Graham *et al.*, 2002a). No work has yet been carried out to assess the efficacy of pre-treated plastic sheeting or canvas or the persistence of the insecticide in a tropical climate.

Insecticide-treated blankets

The use of pyrethroid treated top-sheets (cotton sheets used for cover when sleeping) has been shown to exert deterrent and killing effects on malaria vectors in Pakistan (Rowland *et al.*, 1999). Their efficacy has been demonstrated against transmission of leishmaniasis (Reyburn *et al.*, 2000) and malaria (Rowland *et al.*, 1999). As a result of these studies permethrin treatment of top-sheets is now used operationally for controlling outbreaks of malaria in refugee camps and villages in Pakistan and Afghanistan (Rowland *et al.*, 2004). To date there have been no studies examining the potential of insecticide-treated blankets (as opposed to top-sheets), in terms of their entomological effect, acceptability by users or disease impact.

1.4 Treatment of malaria in complex emergencies

Prompt and effective treatment of malaria episodes is always the priority activity in malaria control. In the acute stage of an emergency, when there may be very high incidence of malaria or the threat of an epidemic, the most appropriate treatment is the most effective. Timely and effective treatment of cases is also important to reduce the parasite reservoir and thereby limit transmission of the disease. Drugs which rapidly clear gametocytes could additionally lower transmission in some environments but would of course need to be efficacious at treating the illness in order to appropriate. Rapid action and the highest possible efficacy are especially important in the acute stage of an emergency when case loads are high and poor access to treatment may prevent return visits of patients failing treatment (Allan, 2001).

1.4.1 Artemisinin-based combination therapy

Combination therapies with artemisinin derivatives (ACT, "artemisinin-based combination therapy") have, in recent years, become considered the most suitable treatment regimens for falciparum malaria where failing first-line mono-therapies need to be replaced. Their use has been widely advocated (Kremsner & Krisner, 2003), in particular the arguments for using only these highly efficacious regimens in emergency settings has been vociferously advocated by Médecins Sans Frontières (MSF, 2003).

The rationale behind the use of a combination of drugs with distinct modes of action is to delay the selection (or progression) of resistance to either of the partner drugs (White *et al.*, 1999), though there was debate as to whether the calculations supporting this theory could predict the true impact on a wider parasite population rather than on individual infections (Bloland *et al.*, 2000).

Artemisinin compounds have been shown to have dramatically rapid action and be highly efficacious in areas of existing resistance to chloroquine (Hein & White, 1993; Olliaro *et al.*, 2001). They are therefore often considered the most appropriate choice where the cheap mono-therapies are failing, purely from a therapeutic point of view (MSF, 2003). Several trials of artemisinin-based combinations have been carried out. A recent meta-analysis examined 16 of these and concluded that ACT has the potential to improve treatment outcomes in areas where conventional treatment is failing (International Artemisinin Study Group, 2004). There are however, no data on the efficacy of ACTs in the Indo-Pakistan sub-continent.

1.4.2 Gametocytocidal drug regimens

The 8-aminoquinolines, of which primaquine is the most used, kill mature falciparum gametocytes (Rieckman *et al.*, 1968, Kamtekar *et al.*, 2004). The 4-aminoquinolines (e.g. chloroquine, quinine) on the other hand, have no effect on the longevity or infectiousness of mature gametocytes (Carter & Graves, 1988), although they may suppress the maturation of young gametocytes. The addition of a single dose of primaquine (45mg) to routine treatment with chloroquine has therefore been recommended in the past for the treatment of falciparum patients in some cases: i) in patients returning to areas where malaria was eradicated and reintroduction was a possibility; ii) during epidemics, and iii) in areas of low transmission where control of disease transmission in this way is feasible (WHO, 1990, 2001b). Co-treatment with primaquine has been shown to result in a reduction of malaria transmission when used in mass treatment campaigns in Nicaragua (Garfield & Vermund, 1983) and through passive case detection in North Sumatra (Matsuoka *et al.*, 1986; Kaneko *et al.*, 1989).

The gametocytocidal activities of artesunate are well documented (Targett *et al.*, 2001; von Siedle *et al.*, 2001; International Artemisinin Study Group, 2004), either as a result of the rapid clearance of trophozoites, or a directly gametocytocidal action (Sutherland *et al.*, 2003). Recently, use of ACTs for epidemic response or in epidemic prone areas has been recommended (WHO, 2004c). It may be that ACTs are a more effective choice than co-treatment with primaquine in situations where transmission reduction through treatment can play a useful role (low transmission settings) or is an urgent need (epidemic response or in epidemic prone settings such as new refugee or IDP camps). A direct comparison of the gametocytocidal effects of these two drugs has not been made and would be useful.

1.4.3 Treatment of vivax malaria

Plasmodium vivax accounts for over half of all malaria outside Africa and is the predominant species in south and central Asia, North Africa, Oceania and Central and South America. In the study setting 80 – 85% of the malaria is attributable to *P. vivax* (Sina, 2002). In the chronic emergency or unstable settings in the Horn of Africa: Sudan, Somalia and Ethiopia, between 5 and 30% of malaria cases may be due to vivax (Newton *et al.*, 1994 (Somalia); Mendis *et al.*, 2001 (Sudan); Mengesha *et al.*, 1999; World Malaria Report, 2005 (Ethiopia)), though data are limited.

For many years, in many countries, the prevailing treatment policy has been to treat infections of either species with a standard dose of chloroquine. However, the rising

and wide-spread chloroquine resistance in *Plasmodium falciparum* means that treatment recommendations for falciparum and vivax malaria are having to diverge in many countries.

Often, especially at the periphery or in settings under-going or recovering from an emergency, differential malaria diagnosis may be poor. Complex emergencies are often characterized by a drain of technically qualified staff, breakdown or absence of infrastructure and non-functioning training or supervisory systems. These factors mean microscopy services, even if previously good, may be absent or poor. Although rapid diagnostic tests (RDTs) with good specificity and sensitivity are now available (WHO, 2000) their stability depends on storage conditions and they are expensive. While funds are likely to be available in acute emergencies or some post-emergency settings for routine RDT use, a national diagnostic policy based on RDTs may be prohibitively expensive; no country has yet made the decision to confront the financial and logistic challenge of putting RDTs in place as routine diagnosis for a national strategy. In many settings, diagnosis remains clinical. Where microscopy is in place there is evidence suggesting that vivax malaria may be more often wrongly diagnosed as falciparum than *vice versa* (Bualombai *et al.*, 2003). Even in areas with relatively good microscopy mixed infections are often missed (Snounou & White 2004) and it appears that a mixed infection is more likely to be reported as a mono-infection of *P. falciparum* rather than a *P. vivax* because the density of *Plasmodium falciparum* is usually much higher and the gametocytes are more obvious.

Cases of vivax malaria resistant to chloroquine, whilst currently rare, are increasing, and may become more of a problem in the future. Confirmed reports (*in vivo*, *in vitro* and molecular) of chloroquine resistant vivax have been reported from Oceania, Asia and South America (Looareesuwan *et al.* 1999; WHO 2001b) and most recently from Peru (Ruebush *et al.*, 2003), Indonesian Papua (Sumawinata *et al.*, 2003), Turkey (Kurcer *et al.*, 2004) and Sri Lanka (Hapuarachchi, *et al.*, 2004).

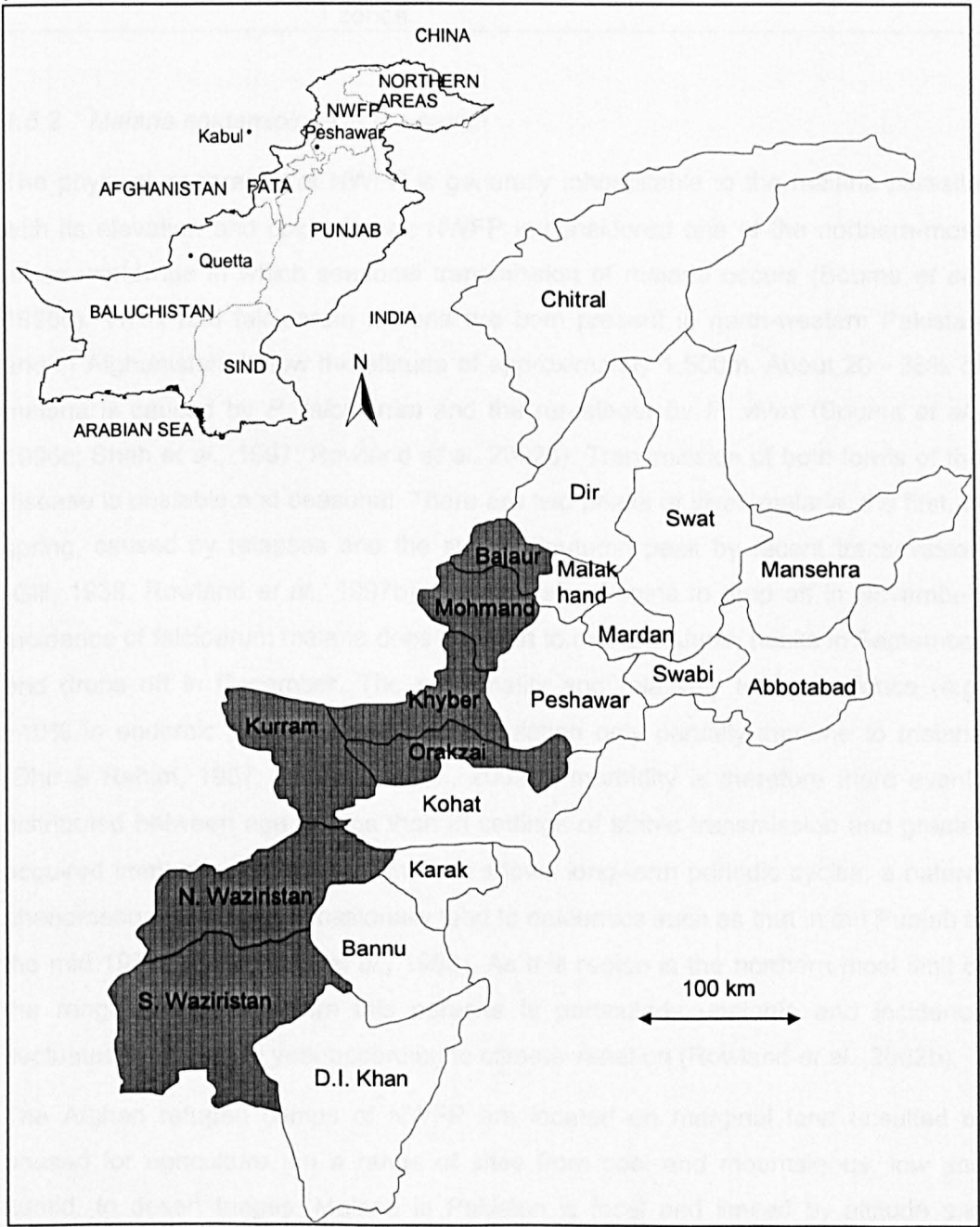
Published data for cure-rates of vivax malaria by SP monotherapy are limited. One study puts the clinical response rate to SP at around 50% (Pukrittayakamee *et al.*, 2000; 2004), citing SP resistance as the cause. It is thought that there may be innate SP resistance of *P. vivax* associated with a sequence polymorphism at the drug-binding site of the target enzymes (Sina, 2002). However it may also be that there is a pre-existence of resistance to antifolate drugs in some areas as a result of the use of these drugs for treatment of falciparum malaria; mutations in the genes

that encode for dihydrofolate reductase thymidylate synthetase appear to cause the vivax resistance (*dhfr* resistance) (de Pecoulas *et al.*, 1998).

1.5 Malaria and malaria control in the chronic emergency setting of NWFP, Pakistan

1.5.1 Geography of the North West Frontier Province

Figure 1-1. Districts of the North-West Frontier Province (NWFP) and location of the province within Pakistan.



[Source: Kolaczinski *et al.*, 2004]

Table 1-2 Characteristics of the North West Frontier Province of Pakistan

Characteristic	Detail
Latitude	Between 32° and 37° north
Borders	South: Baluchistan; East: Punjab and Kashmir; North: China; West: Afghanistan, for approximately 1100km
Altitude	150m in the vale of Peshawar to 3000m in the mountainous north
Population	~17 million and includes ~ 3.5 million Afghan refugees
Population characteristics	85% rural (farming and herding) remainder urban
Cities	Peshawar is the capital of the province and the main urban centre
Climate	Extremely cold winters due to the latitude and altitude. The most populated areas lie in temperate climate zones

1.5.2 Malaria epidemiology in the region

The physical geography of NWFP is generally inhospitable to the malaria parasite with its elevation and cold winters; NWFP is considered one of the northern-most areas worldwide in which seasonal transmission of malaria occurs (Bouma *et al.*, 1996c). Vivax and falciparum malaria are both present in north-western Pakistan and in Afghanistan, below the altitude of approximately 1,500m. About 20 - 35% of malaria is caused by *P. falciparum* and the remainder by *P. vivax* (Bouma *et al.*, 1996c; Shah *et al.*, 1997; Rowland *et al.* 2002b). Transmission of both forms of the disease is unstable and seasonal. There are two peaks of vivax malaria, the first, in spring, caused by relapses and the summer/autumn peak by recent transmission (Gill, 1938, Rowland *et al.*, 1997b). Transmission begins to drop off in November. Incidence of falciparum malaria does not start to rise until June, peaks in September and drops off in December. The seasonality and relatively low prevalence (e.g. ~10% in endemic areas) results in a population only partially immune to malaria (Dhir & Rahim, 1957; Rowland *et al.*, 2002b); morbidity is therefore more evenly distributed between age groups than in settings of stable transmission and greater acquired immunity. In Pakistan, malaria shows long-term periodic cycles, a natural phenomenon which can occasionally lead to epidemics such as that in the Punjab in the mid 1970s (de Zulueta *et al.*, 1980). As this region is the northern most limit of the range of *P. falciparum* this parasite is particularly unstable and incidence fluctuates from year to year according to climate variation (Rowland *et al.*, 2002b).

The Afghan refugee camps of NWFP are located on marginal land unsuited or unused for agriculture, on a range of sites from cool and mountainous, low and humid, to desert fringes. Malaria in Pakistan is focal and limited by altitude and temperature (Bouma *et al.*, 1996c) the type of terrain and the availability of suitable

mosquito breeding sites (Rowland *et al.*, 1997c). Some camps are therefore suited for mosquito breeding and malaria transmission whilst others are not. The majority of camps, however, are situated in relatively low lying regions and those suitable for malaria transmission are characterised by water logging and borrow pits, high water tables, seasonal flooding, nearby rice cultivation and poorly maintained tube wells and water channels (Rowland *et al.*, 2002b).

Reliable estimates of the burden of malaria in Pakistan are hampered by i) the focal nature of the disease; ii) the low levels of transmission dependant on climatic factors resulting in proportionally sizable changes from year to year and iii) the widespread use of private facilities for treatment seeking, cases that go unreported (Donnelly *et al.*, 1997a). A cautious estimate for the country taking into account reported figures and proportional use of the private sector documented in two regional studies gave, in 1997, an annual incidence of malaria cases of 500,000 (Donnelly *et al.*, 1997a).

1.5.3 *The malaria vectors in the region*

There is a large body of literature detailing the anophelines of the Indian sub-continent (including: Christophers, 1933; Ramachandra Rao, 1984). Aslamkhan (1971) lists 25 species of Anophelines from Pakistan. Seven of these are common in NWFP: *An. culicifacies*, *An. stephensi*, *An. subpictus*, *An. nigerrimus*, *An. pulcherrimus*, *An. fluviatilis* and *An. annularis*. Each has been suspected of being a vector at some point in its range (Rao, 1984; Wattal & Kalra, 1961). However, in Pakistan only *An. stephensi* and *An. culicifacies* are considered to be important vectors. No other species were sporozoite positive in any of the early dissection records from the Punjab (Covell, 1931, 1944; Wattal & Kalra, 1961) and studies in the region have shown these other species to associate only rarely with man (Reisen & Boreham, 1979). As a result, these species are often omitted from entomological studies in the area, despite the fact that the vectorial capacity of *An. stephensi* and *An. culicifacies* seems far too low to account for transmission (Reisen & Boreham, 1982).

1.5.4 *Malaria control in the Afghan refugee camps of NWFP*

Since the Soviet occupation of Afghanistan in 1979, Pakistan has played host to Afghan refugees. The changing political situation in Afghanistan over the subsequent decades led to several waves of refugee influx, with intermittent periods of refugee return. The late 1980s and early 1990s saw the peak refugee influx with over 3 million refugees living in Pakistan during these years. The most recent wave

of refugees into Pakistan took place during and after the US-led bombardment of Afghanistan in 2001 which brought about the fall of the Taliban regime. The relative stabilisation since then has led to a gradual return, although the ready permeability of the border makes it difficult to record accurate numbers.

Over three hundred camps were established in NWFP, Baluchistan and the Punjab to accommodate the early influx of refugees, with the majority (75 - 80%) finally settling in the sixty semi-permanent camps in NWFP. Originally refugees were housed in tented villages established by the Pakistani government (Dupree, 1988). Within the camps most families quickly replaced the temporary tents with permanent mud and stone compounds and houses, (Dupree & Dupree, 1988; Bouma & Rowland, 1995) and found casual work to supplement their rations (Christensen & Scott, 1988). Schools, clinics and water supply systems were established (Rowland *et al.*, 2002b). Over time rations were reduced and finally brought to an end in the mid 1990s when refugees were considered self-sufficient at the level of the poorest in Pakistan.

Camps house on average 10,000 people but some larger camps may house over 30,000 (Rowland *et al.*, 2002b). In the last few years (2002 onwards) as assisted repatriation has stepped-up, some of the smaller camps have been closed or merged with larger camps. The camps are administered by UNHCR. UNHCR and its Pakistani Government counterpart, the Project Directorate for Health (PDH), are together responsible for co-ordinating the provision of health-care. A number of UN agencies and non-governmental organizations (NGOs) are involved.

Refugees from Afghanistan were leaving a country which had achieved some success in malaria control prior to the outbreak of conflict. The eradication programme had been initiated in 1958. It made slow but satisfactory progress until 1969 when there was a resurgence of vivax malaria (Wernsdorfer & McGregor, 1988). In 1970, 20,000 cases were reported and by 1972 this figure had risen to 82,000 cases. A number of factors were blamed, including DDT resistance, operational constraints, movements of nomadic tribes and the exophily of local vectors (Gramiccia & Beales, 1988). Nevertheless the malaria situation remained better than in Pakistan with a smaller proportion of cases caused by *P. falciparum*; 118,000 cases were reported in 1982 and according to WHO records, just 0.2% of these were caused by *P. falciparum*. The situation has deteriorated considerably since then and there has been a resurgence of *P. falciparum* in particular. In 1995, laboratories monitored and cross-checked by the Dutch non-governmental

organisation, HealthNet International (HNI) diagnosed 183,000 cases of malaria in the eastern provinces alone, of these 18% were caused by *P. falciparum*.

In the first few years after their arrival there were higher rates of malaria in refugees than in the local population, despite the fact that locals out-numbered refugees by five to one and active case detection (a remnant of the eradication days) was still carried out in Pakistani communities. Record keeping was probably more accurate in the camps, but nevertheless incidence was clearly higher in refugees than in locals. This is likely to have been a result of a combination of lower levels of immunity in the refugee population (Suleman, 1988) as well as the location of the camps resulting in higher levels of man-vector contact than in some of the local settlements (Rowland & Nosten, 2001). In order to counter the surge in malaria in the refugee community, UNHCR and PDH established a control programme based along the lines of the national malaria control programme. Houses and livestock sheds were sprayed with malathion each summer and basic health units (BHUs) in each camp offered diagnosis and treatment. Slides were sent to local field laboratories for diagnosis. First-line treatment was with chloroquine and primaquine. Presumptive treatment was given where diagnosis was likely to take more than one day. At the request of UNHCR the Dutch wing of the medical NGO Médecins Sans Frontières (MSF-H) set up a malaria reference laboratory, a monitoring network and a training centre to ensure satisfactory quality control of microscopy. Wrong reporting was reduced from over 20% to less than 4%.

The successful reduction in malaria in the camps resulted in the use of IRS as a control method becoming increasingly less cost-effective. A potentially more sustainable approach was considered, the use of ITNs. The NGO HealthNet International began ITN projects both in Pakistan and in Afghanistan, where no malaria control activities had been in place since the start of conflict brought an end to IRS and other vertically led interventions. Through subsidized sales, ITN coverage was gradually expanded in two targeted camps in Pakistan, the eastern region of Afghanistan and later in Kabul. Introduction of ITNs into the remainder of the refugee camps in Pakistan was delayed until 2000, as UNHCR and its implementing partners were required to follow official government policy, *i.e.* application of IRS (Kolaczinski *et al.*, 2005). Since 2000 use of ITNs in the camps has expanded, camps with very low malaria transmission are excluded and instead regular data analysis is used to inform rapid, tailored responses to any rise in cases in these camps, using larviciding, treatment of top-sheets or IRS where appropriate.

1.5.6 Anti-malarial drug use in the region

Chloroquine has always been the first-line treatment for both *falciparum* and *vivax* malaria in Pakistan. As a measure to reduce transmission the gametocytocidal primaquine is recommended for co-treatment of *falciparum* malaria. The current level of resistance of *P. falciparum* to the first-line treatment, chloroquine, is alarming. The treatment failure rate has risen from 40% to more than 80% in eastern Afghanistan and neighbouring Pakistan in recent years and resistance appears to be the main reason for the upsurge of this species relative to *P. vivax* (Delfini 1989; Rowland *et al.* 1997b; Shah *et al.* 1997; Rab *et al.* 2001). In all malarious areas examined the *P. falciparum* parasite has demonstrated high levels of chloroquine resistance (Rowland *et al.*, 1997c). Nevertheless there was continued opposition to the idea that chloroquine resistance was widespread and it remains the first-line treatment for malaria (Sultan Ali, 1999).

Chloroquine remains fully effective against *P. vivax* (Rowland & Durrani, 1999; Sultan Ali, 1999.). The recommended treatment regimen for *vivax* malaria is chloroquine alone. The high rate of G6PD deficiency in the population means routine radical treatment with 14 day primaquine is not recommended. A policy of treating with a 5-day course of primaquine as a radical cure, which would be tolerated in G6PD deficient patients, was abandoned after it was shown that this did not result in a significant reduction in the number of relapses compared to patients treated with chloroquine alone (Rowland & Durrani, 1999).

Despite the low levels of malaria in Pakistan and the policy of the National Malaria Control Programme that only patients with microscopically confirmed malaria should be treated it has been reported that blanket treatment of fever patients with antimalarials does take place in many clinics (Hozhabri *et al.*, 2002). With an average slide positivity rate for four provinces as low as 2.7% (National Malaria Control Programme, Pakistan, 1995) it is clear that such treatment practices lead to severe misdiagnosis and incorrect treatment (Hozhabri *et al.*, 2002). Whilst the true prevalence of this practice is not known it is an important consideration when planning treatment policies based on arguments for resistance management and making estimations of costs.

As in many areas of Africa (Greenburg *et al.*, 1989), most malaria cases in Pakistan appear to be treated outside of the government health facilities (Donnelly *et al.*, 1997a). A study in two districts to the north-west of Lahore found that 65% of potential malaria cases made use of private outlets rather than government health

facilities (Donnelly *et al.*, 1997b), a study in south-east Punjab found 89% of respondents reported visiting a private outlet for treatment during the last malaria episode in the household, with 59% of these exclusively using private outlets (32% of respondents reported seeking treatment at more than one facility) (Donnelly *et al.*, 1997a). This situation must be taken into account when considering drug policies based on rationales of reduction of transmission or prevention of further development of resistance.

1.6 Aim and objectives of the thesis

The aim of this thesis was to evaluate options for the control of malaria in complex emergencies which may be suitable for the acute phase and for the post-emergency phase. The current gaps in knowledge and the review of the local situation led to the defining of two general objectives and four specific objectives, outlined below.

General objective 1

To evaluate tools that may be useful both for the individual as well as for a reduction of disease transmission overall.

Interventions which may both be of benefit to the individual (e.g. through rapid relief of symptoms during a malaria episode, or personal protection from malaria carrying mosquitoes) were considered, with a view to how these interventions may also have a wider impact (e.g. the mass effect provided by sprayed shelters or a reduction in transmission of malaria through the use of a drug that reduces the presence of gametocytes in the blood). Such a reduction in overall transmission is a goal that is particularly desirable in the epidemic prone environment of some complex emergency settings.

General objective 2

To evaluate tools that may be useful both locally and globally.

The setting of the experimental work was the Afghan refugee camps on the Afghan / Pakistan border. An objective of this work was to examine interventions that may be both useful to control malaria in the study setting, as well as have a more global usefulness in emergency settings. In this region there are both newly established, tented camps as well as long established camps with refugees who are beginning to return to some areas of Afghanistan. There is therefore a need for a range of vector control interventions that are suitable both settings.

*Specific objective 1**To evaluate insecticide-treated shelter and bedding materials against malaria vectors*

A review of the literature established that there is a dearth of tools proven to provide effective vector control at the early stages of an emergency. Evaluations were planned of potential vector control tools that would pair materials normally distributed at the immediate onset of an emergency (for shelter or warmth) with an insecticide impregnated during manufacture. Evaluations of insecticide-treated plastic sheeting, insecticide-treated tents and insecticide-treated blankets were included, as tools with the potential to provide both personal protection as well as a contribute to a mass reduction in transmission.

The literature review showed that shelters are sometimes sprayed with an insecticide during the acute emergency, although there is evidence for canvas tents sprayed with insecticide providing protection there has been no evaluation of spraying plastic sheeting. This method of treating shelter materials was therefore also investigated.

*Specific objective 2**To evaluate candidate long-lasting insecticide-treated nets against malaria vectors over an extended period of washing*

Insecticide-treated nets are the vector control tool most commonly used in stable settings for malaria control. In a complex emergency there may be limitations to the use of ITNs during the early stages but they are likely to be a useful tool for use in the post-emergency phase when the logistics needs are in place. The advantage of nets being flexible makes them especially beneficial in the post-emergency or chronic stage. The population which health care providers have access to in such environments is often transient; internally displaced people or refugees are likely to return to their areas of origin after stability returns and may move on again elsewhere if this stability proves to be short lived. These populations may move to areas outside the reach of the health services provided by international agencies focusing on defined geographical areas, it is likely that the previous infrastructure and services will have collapsed in the areas to which the displaced are returning. ITNs are a flexible item that returning refugees or IDPs can take with them when they move-on.

A review of the literature demonstrated two areas of particular concern for the use of nets in emergency settings as a tool to provide returning refugees with prolonged protection; i) insecticide-treated nets require retreatment every 6 or 12 months in

order to remain effective as ITNs and ii) only where free mass re-treatment campaigns have taken place have programmes succeeding in ensuring a high proportion of nets remain insecticide-treated.

In the study area retreatment is through free mass campaigns and high coverage is achieved, however increasing numbers of refugees are expected to return to Afghanistan where no retreatment facilities exist. The advent of long-lasting net treatment technologies may be a solution to the question of how to ensure long lasting protection from a treated net. One product has already been shown to be truly long-lasting but has some potential disadvantages; other candidate long-lasting nets in development require evaluation.

Evaluations of two versions of one candidate long-lasting net, and of a second candidate long-lasting net were therefore planned to demonstrate whether these nets could be a useful tool to provide prolonged protection to returning refugees moving outside the reach of re-treatment programmes. During the period of work the most promising of the new long-lasting treated nets was evaluated by several other research teams in other settings against other vectors. In order to gain more evidence for the true longevity of the treatment the results of these additional evaluations were sourced and analysed alongside the data from the field work carried out as part of this thesis.

Specific objective 3

To evaluate artemisinin-based combination therapy for:

- a) its potential to reduce transmission of falciparum malaria in epidemic prone settings such as emergencies*
- b) its efficacy against falciparum malaria in the indo-pakistan sub-continent*

A major current issue facing malaria control in the study setting was (and is) the malaria treatment policy for falciparum malaria. The literature review revealed a glut of evidence for an extremely high level resistance in *Plasmodium falciparum* to the current first-line treatment in this region; it is likely that this is the main reason behind the rise in falciparum malaria. In any setting the priority is always to ensure prompt and effective treatment to individuals presenting with a malaria infection. In an emergency setting, particularly in the acute stage when patient loads are high, effective treatment has the additional importance of ensuring that each episode of illness is successfully dealt with on the first presentation rather than adding to the case load by presenting twice or three times as a result of ineffective treatment. Another result of ineffective treatment is to maintain the reservoir of parasites available for mosquitoes to pick-up, thereby maintaining disease transmission. The

most useful treatment in this study setting, as well as in other emergency settings which occur in areas of low endemicity or areas of high endemicity with a non-immune displaced population, is the regimen which will result in the most rapid cure and the most rapid clearance of sexual parasites.

An evaluation was therefore planned to collect evidence for alternative treatment options for falciparum malaria which would be efficacious in the study setting and would have the potential of lowering transmission in similar settings world-wide.

Specific objective 4

To evaluate a regimen of sulphadoxine-pyrimethamine plus artesunate for its efficacy in the treatment of vivax malaria

In many parts of Asia the bulk of the malaria burden is caused by *Plasmodium vivax*. In the study setting 80% of the malaria is vivax malaria. When emergencies take place in such a setting there is a need to ensure that effective treatment is in place for both diseases. With the declining usefulness of chloroquine for falciparum malaria there is now a divergence in the most effective treatments for the two forms of the disease; chloroquine will remain the first-line treatment for vivax in most areas of the world whilst the guidelines for falciparum malaria move away from chloroquine. In an emergency a common effective treatment policy for malaria has the great advantage of ensuring each episode of illness will be treated effectively regardless of whether differential diagnosis of the type of malaria is attempted or achieved. In this setting, as in numerous others worldwide, an artemisinin-based combination of sulphadoxine-pyrimethamine (SP) plus artesunate (AS) is being considered the most suitable alternative to chloroquine for falciparum malaria. A review of the literature suggests that SP may be less efficacious against vivax parasites than against non-resistant falciparum parasites. It was decided that it would be useful to ascertain the efficacy of SP+AS against vivax malaria in the knowledge that if SP+AS becomes the first-line treatment for falciparum malaria in this region it is likely that numerous vivax infections will be incorrectly treated with SP+AS.

Chapter 2. General methods

2.1 Field Site

All field studies took place in the North West Frontier Province (NWFP) of Pakistan from a base in the province's capital, Peshawar. Work was carried out in collaboration with an international non-governmental organisation (NGO), HealthNet International (HNI), which runs a malaria and leishmaniasis control programme in the Afghan refugee camps of Pakistan and in Afghanistan. The following factors influenced in the choice of this site for the field work:

- Instability and fluctuating security are characteristics of a complex emergency. HNI have worked in the region since 1992 and were therefore well-placed to assist with security advice and logistics.
- Many of the refugee camps in NWFP have existed for over ten years and are well-established. HNI has therefore been able to maintain two long-running entomological research sites in two of these camps. An insectary with established colonies, facilities for bioassay testing and a site for outdoor overnight platform trials exist. Local casual labour is available with experience of this method of overnight testing.
- Entomologists working for HNI are experienced in bioassay techniques, mosquito identification and overnight evaluations at the field site, offering the possibility advice and assistance, if required.
- The site is established as a collaborating centre for WHOPES evaluations making it possible to attract research funding from different sources.
- The United Nations High Commissioner for Refugees (UNCHR) and the inhabitants of these camps welcome the research work HNI carries out as it ensures evidence-based implementation of the NGO's malaria control programme. This creates a stimulating research atmosphere and raises research questions relevant to local health issues.

- The topics considered in this thesis, and the questions answered through field and laboratory studies are of direct relevance to the refugees in this region and those involved in malaria control work there.
- The collaboration with HNI strengthens the chance of findings being quickly incorporated into operational malaria control activities.

The entomological field stations are located in two refugee settlements on the banks of the Kabul River, each approximately 25km from Peshawar. Both settlements have existed for approximately 23 years and have developed from the original tented camps into settlements indistinguishable from the neighbouring Pakistani villages. Inhabitants have constructed traditionally built compounds, mosques and shops using the mud available on-site and purchased wood.

The insectary and bioassay testing site is located within Adizai refugee settlement. The insectary rears a fully susceptible strain of *An. stephensi*. Further details of the insectary colony and conditions are given below in section 2.2. Insectary bioassays and, in one study, overnight platform trials using insectary reared mosquitoes, took place at this site.

The overnight platform assays using wild-caught mosquitoes took place at the entomological field station in Azakhel refugee settlement. Here the land is waterlogged and mosquitoes reach particularly high densities. Five platforms for overnight tests already existed at the site, during the period of field-work further funding was secured and five more platforms were constructed to increase the amount of work feasible during the mosquito season. Further details of the platforms and this testing method are given below in section 2.6.

In both camps the rise in the water table during the spring snow melt and summer monsoon gives rise to innumerable mosquito breeding sites. Mosquito populations begin to rise in April with the majority being culicine species; anopheline densities increase from July. Peak mosquito density occurs in August and density declines in November. Cases of vivax malaria occur from March to November and falciparum malaria from August to December.

2.2 Mosquito rearing

Mosquitoes for insectary bioassays performed in Pakistan were reared in the insectary at one of the entomological field stations. The author supervised but did not undertake the mosquito rearing at this site. Mosquitoes for insectary bioassays performed in London were reared in an insectary housed at the London School of Hygiene and Tropical Medicine. These mosquitoes were reared by the author.

The fully susceptible strain of *Anopheles stephensi*, Beech, was reared and used in both Pakistan and London. This strain originated from Beecham's Laboratory, New Delhi, India and was exported to the LSHTM insectaries. From here eggs were exported to Pakistan for rearing in the HNI insectary.

Pakistan: Insectary conditions in the Pakistan insectary were maintained at $26 \pm 2^{\circ}\text{C}$ and $75 \pm 10\%$ relative humidity. Power-cuts and external conditions caused occasional variation outside these ranges.

Larvae were attended to daily. They were fed on a mixture of fish food and baby food (Farex). The larvae were thinned during the first instar stage into batches of approximately 100 per bowl. Once a bowl contained mainly pupae it was placed inside an adult cage for emergence. For rearing of adults to be used for testing the bowl was removed on the second day to ensure all adults in that cage were of a similar age. Other pupae were placed in stock cages for emergence.

Adults were housed in 30cm wire-framed cubes covered with mosquito netting. 10% glucose solution was provided in feeders and replaced once a week. Blood-meals were given when eggs were required, using live rabbits. A small bowl lined with filter paper and with a small amount of water was provided for egg laying. Illumination was on a daily light: dark cycle of 12:12 h.

London: The temperature of the insectary was kept at $28 \pm 2^{\circ}\text{C}$ and the humidity at $60 \pm 5\%$.

Mosquitoes were reared in the same way described above with the exception of the food provided: larvae were fed on baby food only (Farex) and blood meals were of defibrinated horse blood provided in membrane feeders.

2.3 Mosquito netting

Details of the netting materials used are given in the appropriate chapters (Chapters 7 and 8)

2.4 Insecticides

Permethrin, deltamethrin and alphacypermethrin were used for treatment of chadders in Chapter 9. All other entomological studies were carried out with deltamethrin. The following formulations of insecticide were used:

Permethrin: Imperator 25% EC: Zeneca, Fernhurst, U.K.

Alphacypermethrin: Fendona 10% SC: American Cyanamid, Princeton, NJ, U.S.A.

Deltamethrin: K-Othrin 5% SC: Aventis, Frankfurt, Germany.

2.5 Methods used for treating materials with insecticides

2.5.1 Conventional net treatment

Nets were treated with insecticides in the conventional way for comparison with the candidate long-lasting insecticide-treated nets which were treated during production by the manufacturers. The following procedure was followed for the conventional net treatment:

- The surface area of the netting to be impregnated was calculated.
- A net from the same batch of nets to be dipped was immersed in a known volume of water in a plastic bowl. It was then wrung out and removed from the bowl. The amount of water remaining in the bowl was measured and subtracted from the known initial volume to calculate the exact amount of liquid that would be absorbed by each net.
- The amount of insecticide required for one net was calculated in the following way:

Amount of insecticide in ml =

$$\frac{\text{Target deposit density (mg/m}^2\text{)} \times \text{area of fabric (m}^2\text{)}}{\text{Concentration of the insecticide (mg/m}^2\text{)}}$$

- A bulk solution of dipping mixture was prepared. The volume of solution was calculated as enough to dip all the nets plus 1, the mixture remaining after dipping would be measured to ensure the right amount had been used.

- Nets were treated individually:
 - One net was placed in a plastic bowl, unfolded.
 - A measuring jug was used to add the per-net volume of solution to the bowl, poured over the top of the net.
 - The net was then turned and kneaded for 1 minute to ensure all surfaces of the net had been covered with insecticide.
 - The net was then placed on a plastic tarpaulin to dry for approximately 24 hours.
- Nets were labelled with water resistant ink to indicate the type of treatment and with an ID number
- Once dry, nets were put in plastic bags. The same labelling was repeated on the outside of the bag.
- Between testing nets were stored in cool, dark rooms.

2.5.2 Tarpaulins

For comparison with tarpaulins pre-treated with insecticide during manufacture untreated standard tarpaulins of the same dimension and made from the same plastic were purchased and sprayed with insecticide using Hudson X-pert sprayers. The following procedure was used to spray these tarpaulins.

- The surface area of the tarpaulin to be treated was calculated.
- The amount of liquid required to coat each tarpaulin was calculated in the following way:
 - A Hudson X-pert sprayer was filled with a known volume of water.
 - A tarpaulin was laid out flat on the ground.
 - A staff member experienced in the use of the spray pumps carried out the spraying.
 - The spray-gun was held with the nozzle horizontal and about 5 inches from the surface of the tarpaulin.

- Swathes of insecticide were sprayed from the top to the bottom of the tarpaulin. Each swathe overlapped by about 2 inches. The sprayer walked up and down the unsprayed portion of the sheeting at all times.
 - The time taken to spray each swathe was measured to ensure the volume applied did not vary.
 - Once the whole tarpaulin had been sprayed the remaining volume of water was measured so that the volume required to spray one side of the tarpaulin was known.
- The amount of insecticide required to treat each tarpaulin was calculated using the same method described above for the insecticide treatment of netting.
- A bulk solution of insecticide was made-up to ensure enough insecticide to spray all the tarpaulins required. The water and insecticide requirements were calculated as above for the treatment of netting.
- The tarpaulins were sprayed with the insecticide mixture in the same way as that described above. The spraying was timed for each swathe to ensure consistency of application and the volume of insecticide mixture remaining after all tarpaulins were treated was measured to ensure the correct amount of insecticide mixture had been used.
- After spraying, tarpaulins were left flat to dry in the shade. Where a tarpaulin was to be sprayed on both sides this would take place after the first side had completely dried.

2.6 Tests using adult mosquitoes

WHO recommendations for the testing of bio-efficacy and persistence of insecticides on treated surfaces were followed (WHO, 1998).

Insecticide-treated materials tested were evaluated under WHOPES phase I and phase II criteria. Under the phase I evaluation criteria, bioassays were carried out with insectary reared mosquitoes, under the phase II evaluation criteria, products were tested in small-scale field-trials which took place overnight with wild vectors.

2.6.1 *Insectary bioassays*

Insectary bioassays were carried out using a fully susceptible strain of *Anopheles stephensi* (Beech). Female, non-blood-fed, 2–3 day old mosquitoes were used. Replicate tests were carried out to ensure at least 200 mosquitoes were exposed per sample of material tested. Negative control bioassays were carried out on untreated materials. Bioassay rounds were repeated when negative control mortality was higher than 5% in fixed-time exposure bioassay or any mosquitoes were knocked-down within 30 minutes in median time to knock-down bioassays on untreated material.

Fixed-time exposure bioassays

In fixed time exposure bioassays 8-11 mosquitoes were exposed under WHO cones to pieces of the test material for a fixed period, either 3 or 10 minutes. The test material was pinned to a bioassay board on the wall of the testing room, with the cone pinned to this material.

Following the exposure period the mosquitoes were transferred using a mouth aspirator to a paper cup and held under insectary conditions with access to sugar solution.

The number of mosquitoes knocked-down after one hour and dead after 24 hours was recorded. Mosquitoes were scored as knocked-down if they were seen to be lying on the base of the cup. Before scoring mortality the bottom of the cup was tapped twice from below with a pencil, mosquitoes were scored as dead if they were unable to fly.

Median time to knock-down bioassays

In median time to knockdown bioassays (MTKD) batches of 11 mosquitoes were exposed continuously to the test material and the observed time to knockdown of the median (6th) mosquito was recorded.

Ideally MTKD tests take place in apparatus where the mosquitoes are forced into constant contact with the test material whenever they are not in flight. This was achieved by wrapping the test material around a wire frame: either a wire frame consisting of two intercepting circles about 15cm in diameter, or, in later studies, a wire-framed 10cm cube.

This method was only possible for testing of material that was both flexible enough to be wrapped around such a frame and which mosquitoes can be observed through, such as netting material. For MTKD bioassays on plastic sheeting and blankets this was not possible. In these cases mosquitoes were exposed to the material in a standard WHO test cone for observation of median time to knock down, as used in the fixed-time exposure bioassays.

2.6.2 Overnight outdoor platform tests (Figure 2-1)

Overnight platform tests were carried out following the procedure of Hewitt and Rowland (1999) and Rowland *et al.* (1999). This experimental design emulates the man-vector contact that is experienced during the summer months in this area of Pakistan and neighbouring Afghanistan, when people sleep outdoors in their compound courtyards.

Platforms constructed from rectangular brick walls filled with stone and mud were the site of the overnight tests. Each platform (8m x 6m) is raised approximately 1m from the ground. Along each outside edge of the surface of the platform are moats which were filled with water and detergent to prevent scavenging ants from gaining access to the platform (the detergent breaks up the surface tension which would otherwise allow ants to cross the water). When testing took place a large white sheet was spread over the platform to facilitate collection of mosquitoes in the morning. A large "trap-net" (length 6m x height 2.5m x width 5m) made of untreated mosquito netting was suspended over the platform from four poles embedded in the ground (also with moats) at each corner, a good seal was made between this and the floor-sheet by folding over the floor sheet with the hem of the trap-net two or three times and placing stones on this fold.

On the platforms, within the trap-net, the item being tested (a net, a blanket, a plastic tarpaulin, a canvas tents or a combination of these) was placed (Figure 2-1). Men dressed in local dress (cotton shalwar-chemise), slept under the test item. In some trials a cow was used instead of sleeping men as the vector mosquitoes in this region are highly zoophilic.



Figure 2-1. Overnight outdoor platform, test materials in readiness for a nights testing.

For the first half of the night wild, host-seeking mosquitoes were collected from the outside of the trap-nets and released within. Near to the test site calves were tethered under untreated nets to supplement the number of mosquitoes attracted to the site. The following morning all mosquitoes were collected from within the trap-nets, separated into dead or alive, and kept in humidified cups with sugar solution for a further 12 hours before scoring delayed mortality. All mosquitoes were categorized as blood-fed or unfed, identified to genus level and the anophelines to species level.

End-point indicators used in analysis were: dawn mortality, 24 hour mortality and blood-feeding rate (feeding inhibition). Mortality analysis gives an indication of the potential mass effect on mosquito populations and blood-feeding rate an indication of personal protection.

2.7 Statistical analysis for entomological data

All data were entered in Excel 98 and analysed using either STATA 6 or STATA 7. Statistical analyses were carried out on two types of data: proportions (of mosquitoes dead, knocked down or blood-fed) and times (mean median times to knockdown).

Proportions were analysed in two different ways: in the first two studies carried out (Chapters 3 and 9) arcsine transformation was used; in the other entomological studies blocked logistic regression was used.

- i) *Arcsine transformation:* Proportional data were arcsine transformed to normalise the variance. These data were then subjected to analysis of variance to examine the effect of treatment on blood-feeding and mortality rates. Un-paired t-tests were performed to make specific comparisons. Means and confidence intervals were back-transformed for presentation.
- ii) *Blocked logistic regression:* The total number of mosquitoes in each replicate test (each platform for overnight tests or each replicate of a set of fixed-time exposure bioassays) and the number knocked down, dead or blood-fed were included in a blocked logistic regression model. Comparisons between treatments were made by successively dropping treatments from the overall model. This process allowed each treatment to be compared statistically with every other. Means and confidence limits of the constant for each treatment were back transformed for presentation as follows:

$$x' = \frac{1}{1 + (1/\exp(x))} \quad \text{Where: } x' = \text{back-transformed value}$$

x = the value from the logistic regression

The advantage of blocked logistic regression is that information from each mosquito contributes to the overall analysis rather than just the proportion of mosquitoes from each test (with different denominators each time) being used in the analysis (as in the method of arcsine transforming proportions and then analysing those data).

For ease of comparison between overnight platform trials treatment induced mortality and reduction in blood-feeding were calculated using Abbott's formula (Abbott, 1925):

$$\% \text{ treatment induced mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

For percentage treatment induced reduction in blood-feeding, percentages blood fed replace percentage mortality in the formula and the nominator becomes "% control blood-feeding" rather than "100 – % control blood-feeding".

2.8 Ethical considerations

All protocols used in this thesis were approved by the London School of Hygiene and Tropical Medicine ethical committee. Written approval for studies carried out in Pakistan were provided by the Pakistan Medical Research Council. The ethical clearance documentation is included as Annexe 1.

Chapter 3. An entomological evaluation of insecticide-treated plastic sheeting

3.1 Introduction

Canvas tents sprayed with pyrethroid insecticide are a proven means of controlling malaria epidemics (Hewitt *et al.*, 1995, Bouma *et al.*, 1996a). In recent years plastic tarpaulins have replaced canvas tents as the favoured shelter material for refugees in certain settings, especially when there is a large population requiring shelter. This is because polyethylene sheeting is cheaper to make, cheaper to air-freight, and easier to stockpile (Rowland & Nosten, 2001). If this material could be pre-impregnated with insecticide, be shown to kill malaria vectors, and protect against malaria it would have major advantages, as it would require no additional resources or organisation other than those already deployed at the outset of an emergency. The global malaria control initiative, Roll Back Malaria (Nabarro, 1999), has been working with industry to develop factory-impregnated plastic sheeting (Allan, 2001; Allan & Guillet, 2002; Vestergaard, 2002).

The efficacy of these tarpaulins in laboratory bioassays has already been established by another research group at the London School of Hygiene & Tropical Medicine. There was 100% mortality ($N=50$) in insectary-reared mosquitoes (*An. stephensi* Beech) after just 1 minute of exposure (and 24 hour holding period) in tarpaulin-lined WHO resistance test kits on unweathered sheeting and on “weathered” sheeting, which had been exposed outdoors in London to winter conditions for 84 days.

This chapter describes the first evaluation made under controlled conditions in a refugee camp.

3.2 Materials and Methods

3.2.1 Study location

This study took place at the HealthNet International entomological field station in Azakhel refugee settlement, described in more detail above (Chapter 2, Section 2.1)

3.2.2 Tarpaulins

The physical structure of the tarpaulin is a core-weave matrix (200 μ m thick) covered with two layers of laminate (100 μ m), weighing 200g/m². The core weave is made of

high-density polyethylene and the laminates of low-density polyethylene. Pre-treated tarpaulins and tents made of the same material, dyed white on the outside and black on the inside and impregnated with deltamethrin during manufacture were manufactured by Vestergaard Frandsen (Denmark). The core structure acts as a store for insecticide and the outer layers serve to physically and chemically protect the store and to regulate migration of insecticide to the surface. Owing to their physico-chemical properties, the laminates allow migration of insecticide, which builds up at the surface during storage. The concentration at the surface is a balance between the concentration in the core layer, migration, and inactivation by ultraviolet light (UV). Through appropriate use of migration-minimizing chemicals and UV filters in the two laminates, a more constant effect at the surface is obtained. The concentration of deltamethrin during the mixing process was 45 mg/m² in the surface laminates, and the total concentration was 2g deltamethrin per kg of tarpaulin. Chemical analysis in Gembloux, Belgium, using acetone extraction, showed that 20-30% of deltamethrin was lost in the processing. This is because the operational temperature for tarpaulin production is similar to the evaporation temperature of the insecticide. This analysis is an overall analysis, and does not reveal the final distribution at the surface.

The pre-treated plastic sheeting was compared to standard UNHCR tarpaulins. These have the same physical properties as the plastic sheeting manufactured by Vestergaard Frandsen but are untreated and stained blue on one side and white on the other. These tarpaulins were sprayed with deltamethrin suspension concentrate at 30mg ai/m² using a Hudson X-pert™ sprayer.

3.2.3 Outdoor platform studies

The methodology described above (Chapter 2, section 2.2) was used. The tarpaulins were constructed as Λ -shaped shelters using a ridge-pole and 2 upright poles. The shelters were open at each end and pegged to the floor along the edges. Within each shelter a man clothed in shalwar chemise and covered with a cotton sheet slept on a bedroll on the floor.

The factory-impregnated plastic sheeting was tested against (a) a standard untreated UNHCR plastic sheeting as a control, (b) a UNHCR tarpaulin sprayed with deltamethrin SC at 30mg ai/m² and (c) a deltamethrin factory-impregnated tent (also manufactured by Vestergaard Frandsen). Shelters of each treatment type were tested for one night on each of four platforms in rotation.

Testing took place in November 2001.

3.2.4 Statistical analysis

Data were entered in Excel 98. Statistical analyses were done using Stata 6. Proportional data was arcsine-transformed and subjected to analysis of variance to examine the effect of treatment on blood-feeding and mortality rates. Means and confidence intervals were back transformed for presentation.

3.3 Results

An average of 202 ± 15 (\pm standard error) culicines and 39 ± 7 anophelines were caught on each platform per night. The majority of anophelines were *An. subpictus* (18 ± 5) and *An. stephensi* (15 ± 2), plus small numbers of *An. culicifacies*, *An. fluviatilis*, *An. splendidus*, *An. pulcherrimus* and *An. annularis*. With each individual species of anopheline present only in low numbers, the results were grouped by genera for presentation (Figure 3-1). Tables 3-1 and 3-2 show the mortality and blood-feeding rates for culicines and the two most abundant anophelines. The majority of anophelines on platforms with insecticide-treated tarpaulins or tents died, whereas mortality on the untreated sheeting was never more than 6% (Culicines: $F_{(3, 12)}=33$, $P<0.001$; *An. stephensi*: $F_{(3, 12)}=24$, $P<0.001$; *An. subpictus*: $F_{(3, 12)}=46$, $P<0.001$). There were no significant differences in mortality between the three insecticide-treated shelters. Culicines showed slightly higher survival rates than anophelines. Blood-feeding rates were consistently low throughout the trial for anophelines and for culicines. There were no differences in blood-feeding rate between the insecticide-treated and the untreated shelters (Culicines: $F_{(3, 12)}=0.47$, $P=0.71$; *An. stephensi*: $F_{(3, 12)}=0.3$, $P=0.82$; *An. subpictus*: $F_{(3, 12)}=0.61$, $P=0.62$). Figure 3-1 confirms that the majority of mosquitoes died unfed, presumably before making contact with the host.

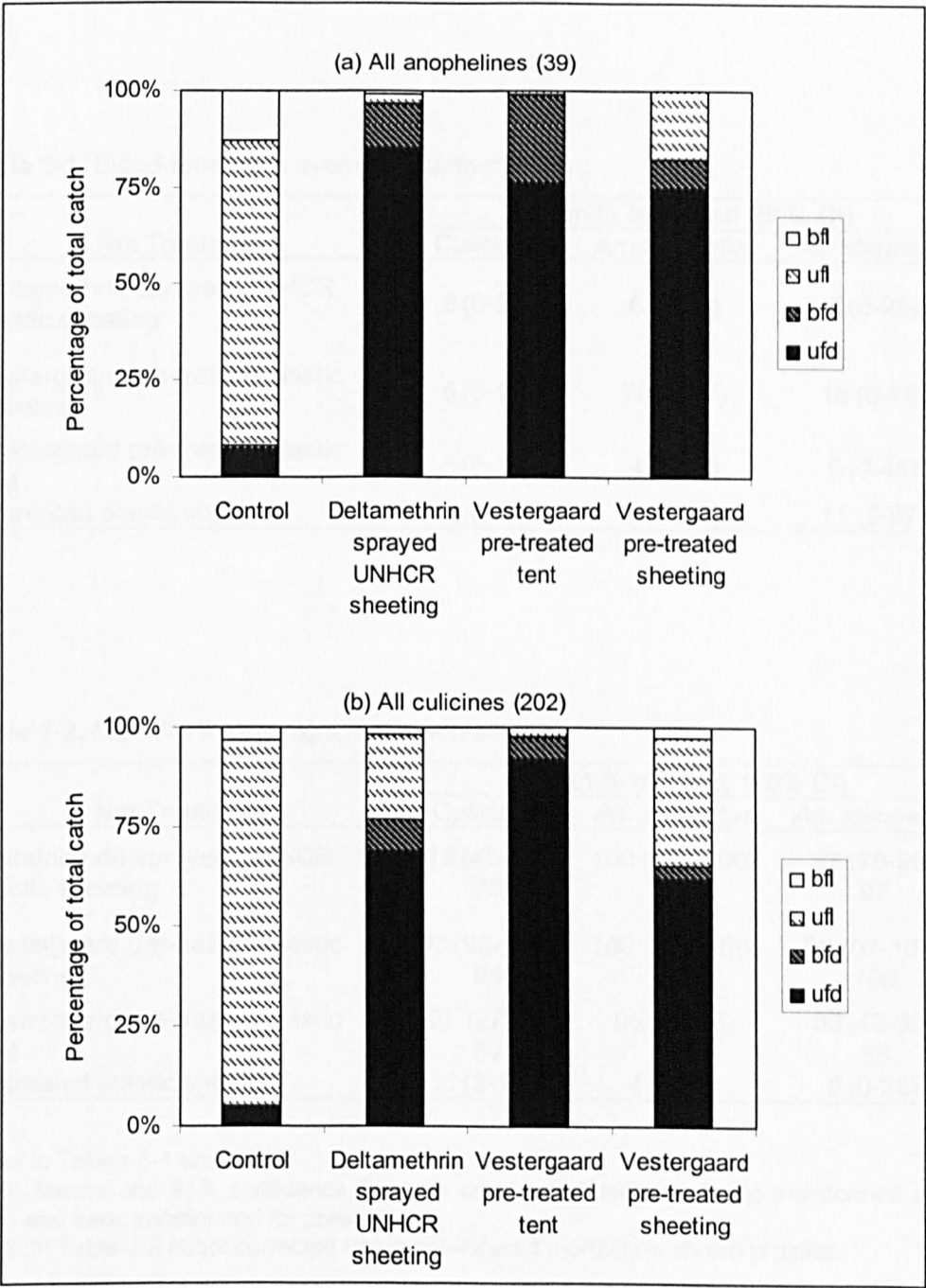


Figure 3-1. Condition of mosquitoes collected from trap nets.

- Notes:
1. Legend abbreviations: bf, blood-fed; uf, unfed; d, dead; l, live.
 2. Number in parentheses is the average number of mosquitoes collected per platform per night.

Table 3-1. Blood-feeding in overnight platform tests

Net Treatment	Mean % blood-fed (95% CI)		
	Culicines	<i>An. subpictus</i>	<i>An. stephensi</i>
Deltamethrin-sprayed UNHCR plastic sheeting	8 (0-29)	6 (0-44)	7 (0-28)
Vestergaard pre-treated plastic sheeting	5 (0-16)	20 (0-77)	18 (0-78)
Vestergaard pre-treated plastic tent	5 (0-19)	4 (0-28)	5 (0-41)
Untreated plastic sheeting	3 (0-9)	6 (0-46)	11 (0-67)

Table 3-2. Mortality in overnight platform tests

Net Treatment	Mean % mortality (95% CI)		
	Culicines	<i>An. subpictus</i>	<i>An. stephensi</i>
Deltamethrin-sprayed UNHCR plastic sheeting	79 (45-99) 78	100 (100-100) 100	97 (78-98) 97
Vestergaard pre-treated plastic sheeting	98 (93-100) 98	100 (100-100) 100	99 (97-100) 100
Vestergaard pre-treated plastic tent	66 (27-96) 64	95 (59-95) 94	86 (43-99) 86
Untreated plastic sheeting	5 (2-10)	4 (0-33)	5 (0-23)

Notes to Tables 3-1 and 3-2:

1. Means and 95% confidence intervals were calculated on arcsine transformed data and back transformed for presentation.
2. In Table 3-2 abbot corrected treatment-induced mortality is shown in italics.

3.4 Discussion

Pyrethroid-impregnated tarpaulins show good potential for malaria prevention in displaced populations. The impressive insecticidal activity demonstrated in laboratory bioassays was corroborated in these field tests in an Afghan refugee camp, where contact between treated material and mosquito was more natural. There was little effect on blood-feeding. This contrasts with the demonstration of feeding inhibition (repellency) that occurred when pyrethroid treated top-sheets (used as a light covering while sleeping) were tested on the same platforms in earlier studies (Rowland *et al.* 1999; Graham *et al.* 2002b; Chapter 6). Crudely erected tarpaulins offer plenty of gaps through which host-seeking mosquitoes may pass *en route* to the host. Thus the potential of treated tarpaulins as a means of malaria prevention may depend upon generating high mortality among the vector population ("mass effect") rather than on direct protection from biting. The prospect for disease control would remain high because coverage in new refugee camps would approach 100% as a result of free distribution of tarpaulins on registering of refugees.

An earlier evaluation of permethrin-sprayed canvas tents in Pakistan showed decay of residue within a few months of the inner surfaces being sprayed (Bouma *et al.*, 1996b). Good insecticide persistence of the factory-impregnated deltamethrin sheeting has been demonstrate by a team at the London School of Hygiene and Tropical Medicine testing sheeting which had been weathered outdoors in London (Graham *et al.*, 2002a). However the timing and location of this exposure (an English winter) means that the sheeting would not have been subjected to particularly intense UV radiation. The UVA and B radiation which accelerates the degradation of insecticides are at higher levels closer to the equator and higher at comparable latitudes in the southern than in the northern hemisphere; cloud cover would also reduce UV levels. It is important that an examination of the resistance of the pre-treated sheeting to weathering also be carried out in a more severe, tropical climate. The London weathering results were encouraging, they demonstrate that the factory-impregnation is able to resist run-off of insecticide on exposure to frequent rain.

While it is important that pre-treated sheeting is able to withstand weathering, the tool will still be useful if the period of residual activity is only a few months. The acute stage of the emergency, when mortality rates are highest, is the period when conventional malaria control is often thwarted by logistic and security constraints

(Rowland & Nosten, 2001). Plastic tarpaulins are distributed during the initial influx of refugees. A few months later, as the camp becomes better established, refugees usually erect their own homes using locally available materials such as mud and straw. Plastic tarpaulins may be retained as useful waterproofing for roofs or walls but may also be put to alternate uses or sold on, and will no longer be useful as a vector control tool (Graham, 2004b). The insecticidal activity of the tarpaulin need only last as long as IDPs and refugees are using the tarpaulins as their main shelter. Once the camp moves into a chronic stage, conventional methods of malaria control (e.g. ITN, IRS) are more easily applied.

Diarrhoeal diseases are often the most important cause of mortality in refugee camps (Toole and Waldman, 1997). The significant role of houseflies in the transmission of some diarrhoeal diseases (Cohen *et al.*, 1991; Chavassee *et al.*, 1999) indicates that the potential of pyrethroid treated sheeting to reduce housefly numbers should be examined. Leishmaniasis is another vector borne disease that can be controlled by residual spraying (Pandya, 1983; Vioukov 1987; Reyburn *et al.*, 2000). Insecticide-treated sheeting therefore has potential as a wider public health tool against a variety of vector borne diseases in refugee camps, alongside its promise as a tool against malaria in the problematic acute phase.

Chapter 4. Further entomological evaluations of insecticide-treated plastic sheeting

4.1 Introduction

Plastic sheeting (or polyethylene tarpaulins) is a shelter material often distributed to provide short-term cover when refugee or IDP camps are first established. Although canvas tents sprayed with insecticide have proved effective at controlling malaria (Bouma *et al.*, 1996a), earlier formulations of insecticides (e.g. wettable powders) were not adhesive to plastics meaning spray campaigns were not an option in a refugee camp using plastic sheeting. In recent years two options for treatment of plastic sheeting with insecticide have arisen (i) newer formulations of pyrethroids, such as suspension concentrate (SC), which may adhere better to polyethylene and allow the possibility of spray campaigns on plastic sheeting and (ii) the global malaria control initiative, Roll Back Malaria (Nabarro, 1999), has fostered public-private collaborations between academia and industry to develop factory-impregnated insecticide-treated plastic sheeting (ITPS) (Allan, 2001; Allan and Guillet, 2002; Vestergaard, 2002). A short study evaluating sheeting sprayed with deltamethrin SC and a prototype plastic sheeting pre-treated with deltamethrin demonstrated an equivalent efficacy; both shelters caused high mortality in host-seeking malaria vectors and nuisance biting culicine mosquitoes, though there was no reduction in blood-feeding (Graham *et al.*, 2002a; Chapter 3).

The benefits of a factory-based insecticide treatment process are two-fold. Firstly, a vector control tool would be in place as soon as shelter materials start to be used, thereby circumventing the logistic and organisational requirements of a spray campaign. Secondly, the insecticide treatment in factory-impregnated sheeting may have a longer residual activity than a spray-on treatment.

Several questions remained unanswered after the previous trial (Chapter 3), relating to the design of a final pre-treated product, the best strategy for spraying insecticide, and the longevity of the pre-treated and hand-sprayed products. Several studies took place in refugee camps in North West Pakistan to address some of these questions. Study 1 examined whether insecticide needs to be bio-available on both the inside and outside surface of a shelter in order to have a killing or repellent effect on host-seeking mosquitoes. If the insecticide-treated plastic sheeting acts in a similar way to indoor residual spraying it may only be necessary to treat the "inner" surface of the sheeting. Only having to treat the inner surface would reduce

the costs of pre-treated plastic sheeting (although there would then be a need to include instructions as to which way the tarpaulin should be oriented). The cost of insecticide spray campaigns would also be reduced as well as the time needed to complete the campaign. The next studies examined the life-span of insecticide on the sheeting: Study 2 concentrated on hand-sprayed sheeting, and compared sheeting sprayed with deltamethrin in suspension concentrate and wettable powder formulations over a period of weathering; Study 3 examined the insecticidal persistence of the prototype pre-treated sheeting in comparison to the most persistent hand-sprayed formulation. In order to inform the product development process the persistence of insecticide on the inner surface of the pre-treated shelter was tested on shelters weathered with either the black side (which incorporates UV filters) facing outermost or white side (which may reflect UV light) outermost. Study 4 and Study 5 evaluated the final pre-treated product developed by the company and marketed as Zerofly™. Insectary bioassays and outdoor overnight platform assays were used to test this product for its efficacy against mosquitoes (study 4) and the persistence of the insecticide treatment (study 5).

It is possible that insecticide-treated shelters such as these may be effective against a broader range of diseases than malaria. In refugee camps diarrhoeal diseases are often the most important cause of mortality (Toole and Waldman, 1997). The significant role of houseflies in the transmission of some diarrhoeal diseases (Chavasse *et al.*, 1999; Cohen *et al.*, 1991) indicates that the potential of pyrethroid treated sheeting to reduce housefly numbers should be examined. In Study 6 house fly mortality and resting behaviour were examined on exposure to treated sheeting. Following a successful first round of tests showing good mortality on exposure to freshly treated sheeting a second series of tests the following year examined housefly mortality on exposure to unweathered sheeting, sheeting that had been weathered for 6 months and untreated sheeting. In order to determine whether house flies may be diverted from resting on the walls or roof of an insecticide-treated shelter onto inhabitants and food within, this second round of testing also examined the resting behaviour of house flies on and near the shelters.

4.2 Materials and Methods

4.2.1 Study location

This study took place at the HealthNet International entomological field station in Azakhel refugee settlement, described in more detail above (Chapter 2, Section 2.1)

4.2.2 Tarpaulins

The prototype pre-treated sheeting tested was the black and white tarpaulin described in Chapter 3, Section 3.2. The final product, marketed as Zerofly™ was made to the same specifications and using the same technology but was dyed a uniform dark blue colour, a dye which incorporated a UV filter.

The pre-treated plastic sheeting was compared to standard tarpaulins such as those used by UNHCR. These have the same physical properties as the plastic sheeting manufactured by Vestergaard Frandsen but are untreated and stained blue on one side and white on the other. These tarpaulins were either left unsprayed as an untreated control or were sprayed with either deltamethrin suspension concentrate (SC) or deltamethrin wettable powder (WP) at 50mg ai/m² using a Hudson X-pert™ sprayer.

4.2.3 Summary of studies

Study 1: Which surface(s) of a sheeting should be treated: the inside, outside or both sides?

- Overnight platform trials of sheeting sprayed with deltamethrin SC 50mg ai/m² either on the inside surface alone, the outside surface alone or both inside and outside surfaces, compared to untreated sheeting.
- These tests took place in October 2002.

Study 2: A comparison of the persistence of two deltamethrin formulations hand-sprayed onto plastic sheeting.

- Insectary bioassays and overnight platform trials of sheeting hand-sprayed with deltamethrin SC or deltamethrin WP, before weathering and after 3 months of weathering, compared to untreated sheeting.
- These tests took place in August 2002.

Study 3: An evaluation of the persistence of insecticide on the prototype pre-treated sheeting.

- Insectary bioassays and overnight platform trials of the prototype pre-treated sheeting before and after weathering (with either the black or white side facing outwards) in comparison to hand-sprayed sheeting (deltamethrin SC) and untreated sheeting. Overnight platform trials were carried out twice, once with human sleepers and once with a calf tethered in each shelter as bait.
- Overnight outdoor tests took place in August and October 2002 with insectary bioassays taking place (after a further weathering period) in March 2003.

Study 4: An evaluation of the efficacy of the final pre-treated product, Zerofly™.

- Insectary bioassays and overnight platform trials of Zerofly sheeting compared to untreated sheeting.
- This testing took place in August 2003.

Study 5: An evaluation of the insecticidal persistence of Zerofly sheeting over 10 months of weathering.

- Insectary bioassays and overnight platform trials of Zerofly sheeting unweathered or weathered for periods up to 13.5 months.
- This testing took place in November 2004

Study 6: An evaluation of the effect of pre-treated sheeting on house fly mortality and resting behaviour in free-flying platform assays.

- First round of tests: measuring mortality in day-time platform studies of pre-treated sheeting compared to untreated sheeting in April 2002.
- Second round of tests: measuring resting behaviour and mortality in day-time platform studies of pre-treated sheeting unweathered or weathered compared to untreated sheeting. These studies took place in April 2003

4.2.4 Weathering of plastic sheeting

In order to evaluate the persistence of the insecticide treatment on the pre-treated and hand-sprayed plastic tarpaulins, these were “weathered” for varying periods. An exposed area in Adizai refugee camp, at the entomological field site of HealthNet International was the location of the weathering. Tarpaulins were erected as Δ -shaped shelters made from the plastic sheeting, a ridge pole and 2 upright poles; the sheets were open at the ends and pegged to the floor along the edges. These tarpaulins were all erected with the apex lying on a north-south line. 25cm x 25cm

squares of sheeting were cut from each tarpaulin to be tested by insectary bioassay, these pieces were all cut from the west facing side of the shelter.

4.2.5 *Contact bioassays*

Fixed-time exposure bioassays for 3 minutes were performed using the methodology described above (Chapter 2, Section 2.2). Where bioassays were carried out on sheeting weathered for varying intervals (*i.e.* in Studies 2, 3 and 5) sections of sheeting were cut from the sheeting at each interval and stored in aluminium foil under refrigeration. At the completion of the weathering period all pieces were bioassayed in one block of bioassays. Replicate tests were planned to ensure one piece of each treatment type and weathering interval would be tested using the same batch of mosquitoes on the same day, with the second replicates taking place the next day *etc.*

Although bioassays with a longer exposure period (10 or 30 minutes) are often used to examine the efficacy of indoor residual spraying, and therefore possibly suitable in these tests, it was decided to use a 3-minute exposure period here. Preliminary tests on the plastic sheeting by another research group at LSHTM had shown 100% mortality in mosquitoes exposed to the sheeting for just 1 minute (Yates, *Pers. Comm*; Graham *et al.*, 2002a). With 30-minute exposure periods these bioassays would not be able to pick-up a gradual decline in efficacy over a period of weathering. However in order to allow some cross-study comparison 10-minute exposure bioassays were carried out here in Study 6 on Zerotfly.

In Study 2 median time to knock down bioassays were also performed, following the procedures described in above (Chapter 2, Section 2.2).

4.2.6 *Overnight platform studies*

The methodology described above (Chapter 2, section 2.2) was used with the modifications as described in Chapter 3, section 3.2. In study 3 (the examination of the persistence of the prototype pre-treated sheeting to weathering) the overnight trial took place twice. In the first round of tests men slept in the shelters as previously described; in the second round of tests a calf was tethered inside each shelter to act as the bait to the host-seeking mosquitoes. The reason for performing some trials with calves rather than sleeping men as bait is due to the highly zoophilic nature of the mosquitoes in this region. Using cows as bait may attract more mosquitoes into a shelter which is important when trying to show the relative

efficacy of two or more different shelters, especially when such differences may be marginal, such as in shelters weathered for only a 3 month interval between tests. Calves were also used in study 6, an examination of the persistence of Zerofly after weathering.

Each year studies took place throughout the summer mosquito season. Over this mid-July to mid-November period ambient temperature and humidity change; there are also differences from year to year. This may affect mosquito behaviour, natural mortality and susceptibility to insecticides. As a reference point for later discussion the month and year of testing is indicated below the results table.

In each study all types of treated (or untreated) shelter were tested concurrently. Each shelter was tested on each night and shelters were rotated around the test platforms with sleepers, bait cows and collecting teams all remaining fixed to one platform.

4.2.7 Day-time free-flying platform tests with *Musca domestica*

Platforms and shelters as described above (Chapter 3, section 3.2) were used for testing the plastic sheeting against house flies, *Musca domestica*. Within each shelter two large bowls of sugar (brown, unrefined) and water mixture were placed to simulate the presence of food in an inhabited shelter. Outside each shelter on the platform a large cardboard box was placed on its side (with the opening on the side rather than on the top), this provided an alternative area of shade.

House fly trials were carried out during daylight hours using wild *M. domestica* which had been collected using sweep nets in the local house compounds no more than one hour prior to the start of the test. The house flies were held in large mesh cages during the collection process.

In the first round of testing in 2002 house flies were released at 11am into the giant trap nets covering the platforms. Four hours later the flies were re-collected (using forceps, mouth aspirators and sweep nets). Live flies were held for a further 24 hours before scoring mortality. Collections were identified and *M. domestica* scored for knockdown and 24 hour mortality. In the second round of testing, in 2003, which examined both resting behaviour and mortality on weathered sheeting house flies were released into the giant trap net for 1 hour only with tests taking place between 11.30am and 3.30pm. Resting positions of house flies were recorded at the following time points after the time of release (0 minutes): 1, 2, 3, 4, 5, 10, 20, 40, 60 minutes. After 60 minutes all flies were re-collected with live flies held for a further 24 hours

before scoring mortality. Collections were identified and *M. domestica* scored for knockdown and 24 hour mortality.

On each day of testing both types of shelter were tested simultaneously. The person recording the resting sites alternated between each type of treatment.

4.2.8 Statistical analysis

Proportional data (knockdown, mortality and blood-feeding) from the platform trials and the fixed-time exposure bioassays were analysed using blocked logistic regression (STATA 6 software).

Comparisons between treatments were made by successively dropping treatments from the overall comparison. This process allowed each treatment to be compared with every other. Means and confidence limits of the constant for each treatment were back transformed for presentation as follows:

$$x' = \frac{1}{1 + (1/\exp(x))}$$

Where: x' = back-transformed value
 x = the value from the logistic regression

For ease of comparison between trials with different baseline mortality or blood-feeding results (on untreated materials) treatment induced mortality and treatment induced reduction in blood-feeding were calculated using Abbott's formula.

N.B. It was intended that the persistence of insecticide after weathering on plastic sheeting would be tested using high pressure liquid chromatography (HPLC). While this technique can be successfully used to determine the quantities of insecticide in fibres of bed nets it was not successful for the ITPS. The acetone extraction method was unable to extract reproducible amounts of deltamethrin from within the polyethylene as the polyethylene was insufficiently or non-uniformly broken down. As a result the deltamethrin quantified could not be reliably identified as the surface deposit of bio-available insecticide or the insecticide held stored in the polyethylene core. With this inability to break down the plastic consistently it was impossible to determine whether the tests measured all of the insecticide from the core, some of it or none of it.

4.3 Results

Study 1: Which surface(s) of a shelter should be treated: the inside, outside or both sides?

Each of the treated shelters tested resulted in higher mosquito mortality than in the untreated shelters, both for culicines and anophelines (Table 4-1). The shelter treated on the inside surface resulted in significantly lower anopheline and culicine mortality than the shelters treated either on both sides ($P = 0.001$) or on the outside only ($P < 0.001$); when the outside or both sides of the shelter are treated, both culicine and anopheline treatment induced mortality is more than double that seen on shelters treated on the inside only. Interestingly, for culicines the greatest treatment induced mortality is seen on the shelter with only the outside treated.

Culicine blood-feeding is reduced in the presence of all treated shelters from that seen on the untreated shelter and is lowest when both surfaces of the shelter are treated (Table 4-2). The shelter treated on the inside surface only has the least impact on culicine blood-feeding. Although statistically significant these differences are small. No effect on anopheline blood-feeding is seen; the percentage of blood-fed anophelines is similar on the three treated shelters and the untreated shelter (Table 4-2).

So, whilst shelters treated only on the inside surface do increase mosquito mortality and reduce culicine blood-feeding over that seen on untreated shelters, they do so to a lesser extent than shelters which are treated only on the outside surface or on both surfaces. The normal behavioural process expected to lead to mortality in treated shelters is the same process that makes indoor residual spraying so effective: mosquito enters, feeds on the host and then rests on the insecticide-treated walls to digest the blood-meal, picking up a lethal dose of insecticide (or, occasionally, rests on the wall prior to taking a blood meal). In contrast, in this study mosquitoes are either a) picking up a lethal dose from the outside of the shelter prior to feeding (a concept which would support the observed reduction of culicine feeding in the presence of shelters treated on the outside) or b) feeding and then resting on the outside of the shelter to digest the blood meal and thence picking up a lethal dose of insecticide (which would support the observation of no reduction in anopheline feeding and higher mortalities in the shelters with insecticide surface outermost). However, the majority of mosquitoes do not take a blood meal, regardless of whether the shelters are treated or untreated.

Table 4-1. 24 hour mortality of mosquitoes on sheeting sprayed on different surfaces in overnight platform trials with human sleepers in October 2002.

	Number of replicate nights	Culicines			Anophelines ⁵		
		Number of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality	Number of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality
Deltamethrin SC - inside surface	9	631.8 (266.9)	12.1% ^a (11.2 - 12.9)	6.7%	45.8 (22.8)	21.1% ^a (17.4 - 25.3)	11.0%
Deltamethrin SC - outside surface	9	597.6 (207.0)	27.2% ^b (26.1 - 28.5)	22.7%	45.2 (24.1)	33.2% ^b (28.8 - 37.9)	24.7%
Deltamethrin SC - both surfaces	9	573.2 (180.8)	21.8% ^c (20.6 - 22.9)	17.0%	44.7 (19.8)	31.6% ^b (27.2 - 36.3)	22.9%
Untreated sheeting	18	672.3 (175.0)	5.8% ^d (5.4 - 6.2)	-	40.5 (17.8)	11.3% ^c (9.2 - 13.8)	-

Notes

1. Percentage dead and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model.
2. Within each genera, rows not sharing a superscript letter differ significantly (P<0.05) in the blocked logistic regression model.
3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used, wild caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included merely to indicate the size of the denominator for calculation of percentages.
4. Due to low numbers of individual species all anophelines have been grouped.

Table 4-2. Blood-feeding of mosquitoes on sheeting sprayed on different surfaces in overnight platform trials with human sleepers in October 2002.

	Number of replicate nights	Culicines			Anophelines ⁵		
		Number of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood-feeding	Number of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood-feeding
Deltamethrin SC - inside surface	9	631.8 (266.9)	5.1% ^a (4.6 - 5.7)	29.2%	45.8 (22.8)	6.1% ^a (4.1 - 8.8)	No reduction
Deltamethrin SC - outside surface	9	597.6 (207.0)	3.2% ^b (2.8 - 3.7)	55.6%	45.2 (24.1)	5.4% ^a (3.6 - 8.1)	No reduction
Deltamethrin SC - both surfaces	9	573.2 (180.8)	2.3% ^c (1.9 - 2.7)	68.1%	44.7 (19.8)	5.2% ^a (3.4 - 7.9)	No reduction
Untreated sheeting	18	672.3 (175.0)	7.2% ^d (6.7 - 7.6)	-	40.5 (17.8)	5.2% ^a (3.8 - 7.1)	-

Notes

1. Percentage blood-fed and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model.
2. Within each genera, rows not sharing a superscript letter differ significantly ($P < 0.05$) in the blocked logistic regression model.
3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used, wild caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included merely to indicate the size of the denominator for calculation of percentages.
4. Due to low numbers of individual species all anophelines have been grouped.

Study 2: A comparison of the persistence of two insecticide formulations hand-sprayed onto sheeting.

Before any weathering the plastic tarpaulins sprayed with either deltamethrin SC or deltamethrin WP gave similar results. Both treatments resulted in higher mosquito mortality than the untreated shelters with treatment induced mortality ranging from 11.3 - 17.2%. Culicine treatment induced mortality was lowest on the SC unweathered sheeting. For anophelines there was no difference between the two (Table 4-3). These unweathered tarpaulins also reduced both anopheline and culicine blood-feeding to about 50 - 54% (Table 4-4).

Deltamethrin SC after weathering

On the 3 months weathered SC-treated sheeting anopheline mortality remained similar and culicine mortality was slightly higher than the mortality seen on the unweathered SC-treated sheeting (16.6% versus 11.3% induced mortality). Anopheline blood-feeding was also similar on the 3 month weathered SC-treated sheeting to the unweathered sheeting, still an almost 50% treatment induced reduction in blood-feeding. However, on the weathered SC-treated sheeting the treatment induced reduction in culicine blood-feeding was minimal (14.2%).

Deltamethrin WP after weathering

On the WP-treated sheeting treatment induced anopheline mortality was similar after 3 months weathering (12.6%) to that on unweathered sheeting (13.5%). The impact on culicine mortality was worse after 3 months weathering with only 7.6% induced mortality compared to the 17.2% on unweathered sheeting. The WP-treatment weathered for 3 months was significantly less efficacious at preventing blood-feeding than similar unweathered sheeting; treatment induced reduction in culicine blood-feeding fell from 48.1% on unweathered sheeting to 14.2% on weathered sheeting. There was no significant reduction in anopheline blood-feeding on weathered WP-treated sheeting.

Sheetings treated with these formulations and weathered for monthly intervals up to 3 months were tested by insectary bioassay. The sheeting treated with deltamethrin WP that was cut out and stored when freshly treated resulted in poor mortality for a freshly treated material (<80%) in the 3 min exposure bioassay (Table 4-5), and took longer to knock-down the mosquitoes than the equivalent sheeting sprayed with deltamethrin SC (Table 4-6). It may be that the wettable powder is more easily brushed or rubbed off the polyethylene sheeting once dry and some of the loading dose may therefore have been lost whilst it was stored in aluminum foil.

The fixed-time exposure tests show a similar decline in efficacy of the two treatments over the weathering period with both resulting in poor (~50%) mortality after 3 months of weathering (Table 4-5). However, this decline in efficacy of the SC formulation observed here does not translate to a decline in mosquito mortality on the 3-month weathered SC-sprayed sheeting in the overnight platform trials.

The median time to knockdown tests give some indication of a difference in the persistence between formulations which corresponds to the differences seen in overnight outdoor assays. Mosquitoes exposed to sheeting treated with the SC formulation are consistently knocked down faster than the WP-treated sheeting weathered for the same period. After 3 months of weathering there is a considerable difference between the two treatments with mosquitoes knocked down on the SC treatment in approximately half the time of those exposed to the WP treatment.

Taking both the MTKD and the overnight data into consideration it appears that deltamethrin wettable powder persists less well than deltamethrin suspension concentrate on plastic sheeting.

Table 4-3. 24 hour mortality of mosquitoes on sheeting sprayed with different formulations of insecticide, unweathered or weathered for 3 months, in overnight platform trials with human sleepers in August 2002.

	Number of replicate nights	Culicines			Anophelines ⁵		
		Number of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality	Number of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality
Deltamethrin SC unweathered	15	345.6 (103.9)	20.8% ^a (19.7 - 21.9)	11.3%	16.8 (18.7)	34.9% ^{ab} (28.3 - 41.0)	17.1%
Deltamethrin WP unweathered	15	401.1 (121.6)	26.1% ^b (25.0 - 27.2)	17.2%	18.7 (17.6)	32.1% ^{ab} (26.9 - 37.8)	13.5%
Deltamethrin SC weathered for 3 months	15	351.3 (110.8)	25.5% ^b (24.3 - 26.7)	16.6%	23.3 (27.6)	39.1% ^a (34.2 - 44.4)	22.4%
Deltamethrin WP weathered for 3 months	15	348.5 (123.3)	17.5% ^c (16.5 - 18.5)	7.6%	22.5 (24.5)	31.4% ^b (26.6 - 36.5)	12.6%
Untreated sheeting	15	365.8 (130.4)	10.7% ^d (9.9 - 11.5)	-	22.3 (19.2)	21.5% ^c (17.4 - 26.2)	-

Notes:

1. Percentages dead and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model.
2. Within each genera, rows not sharing a superscript letter differ significantly (P<0.05) in the blocked logistic regression model.
3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used, wild caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included merely to indicate the size of the denominator for calculation of percentages.
4. Due to low numbers of individual species all anophelines have been grouped.

Table 4-4. Blood-feeding of mosquitoes on sheeting sprayed with different formulations of insecticide, unweathered or weathered for 3 months, in overnight platform trials with human sleepers in August 2002.

	Number of replicate nights	Culicines			Anophelines ⁵		
		Number of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood-feeding	Number of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood-feeding
Deltamethrin SC unweathered	15	345.6 (103.9)	6.4% ^a (5.8 - 7.2)	39.6%	16.8 (18.7)	15.1% ^a (11.2 - 20.0)	53.5%
Deltamethrin WP unweathered	15	401.1 (121.6)	5.5% ^b (5.0 - 6.2)	48.1%	18.7 (17.6)	16.1% ^a (12.2 - 20.8)	50.5%
Deltamethrin SC weathered for 3 months	15	351.3 (110.8)	9.4% ^c (8.6 - 10.2)	11.3%	23.3 (27.6)	16.6% ^a (13.0 - 20.8)	48.9%
Deltamethrin WP weathered for 3 months	15	348.5 (123.3)	9.1% ^c (8.4 - 10.0)	14.2%	22.5 (24.5)	32.2% ^b (27.5 - 37.4)	0.9% n.s.
Untreated sheeting	15	365.8 (130.4)	10.6% ^d (9.8 - 11.4)	-	22.3 (19.2)	32.5% ^b (27.7 - 37.7)	-

Notes:

1. Study 2 took place in August and used human sleepers within the shelters.
2. Percentage blood-fed and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model.
3. Within each genera, rows not sharing a superscript letter differ significantly (P<0.05) in the blocked logistic regression model.
4. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used, wild caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included merely to indicate the size of the denominator for calculation of percentages.
5. Due to low numbers of individual species all anophelines have been grouped.

Table 4-5. Fixed-time insectary bioassays on sheeting sprayed with two different formulations of deltamethrin and weathered for varying lengths of time: mosquito mortality after 3 minute exposure.

Treatment Type	Unweathered	1 month weathering	2 months weathering	3 months weathering
% mortality, (95% CI)				
Deltamethrin SC 50mg ai/m ²	89% (74 - 98)	92% (76 - 100)	78% (60 - 92)	50% (35 - 66)
Deltamethrin WP 50 mg ai/m ²	76% (64 - 85)	63% (42 - 82)	51% (33 - 68)	46% (22 - 71)

Notes:

1. Mortality on untreated sheeting as a negative control was never greater than 5%.
2. All tests were carried out in one batch of bioassays after the 3 months weathering period. Pieces of sheeting cut before weathering and after 1 or 2 months weathering had since been stored wrapped in aluminium foil in a refrigerator.
3. Six replicate tests using 10 or 11 mosquitoes were carried out on each treatment type for each period of weathering: 3 pieces of sheeting were each tested 2 times.

Table 4-6. Median time to knockdown bioassays on sheeting sprayed with two different formulations of deltamethrin and weathered for varying lengths of time: median time to knockdown, seconds (95% CI)

Treatment Type	Unweathered	1 month weathering	2 months weathering	3 months weathering
Deltamethrin SC 50mg/m ²	389 (357 - 422)	456 (382 - 531)	520 (469 - 571)	570 (401 - 740)
Deltamethrin WP 50 mg/m ²	464 (380 - 549)	543 (471 - 615)	663 (573 - 751)	1098 (807 - 1388)

Notes:

1. On untreated sheeting tested as a negative control no mosquitoes were knocked down in a 30 min (1800 seconds) test period.
2. All tests were carried out in one batch of bioassays after the 3 months weathering period. Pieces of sheeting cut before weathering and after 1 or 2 months weathering had since been stored wrapped in aluminium foil in a refrigerator.
3. Six replicate tests using 11 mosquitoes were carried out on each treatment type for each period of weathering: 3 pieces of sheeting were each tested 2 times.

Study 3: An evaluation of the insecticidal persistence of the prototype pre-treated sheeting.

The prototype pre-treated sheeting was evaluated for insecticidal persistence after 3 or 6 months of weathering, the first round of testing took place in August with human sleepers and the second round in October with calves as bait. All pre-treated sheeting, weathered or unweathered and in both rounds of testing, resulted in higher mosquito mortality than the untreated sheeting (Table 4-7). A decline in the efficacy of the pre-treated sheeting at each weathering point was observed, with treatment induced mosquito mortality lower after 3 months weathering than on the unweathered sheeting and falling again, to less than one third of the treatment induced mortality on the unweathered sheeting after just 6 months.

When compared to sheeting hand-sprayed with the most persistent deltamethrin formulation from the previous trial, deltamethrin SC, there is some evidence that the pre-treatment may be more persistent. For culicines, though statistically significant, the difference in treatment induced culicine mortality on the two treatments weathered for 3 months is slight, however, for anophelines similar treatment induced mortality is seen on hand sprayed sheeting weathered for 3 months as that on pre-treated sheeting weathered for 6 months.

All treated shelters (both unweathered and weathered) resulted in some reduction in blood-feeding though those trends that were apparent were more distinct in the trial where calves were used as bait (Table 4-8). In this trial treatment induced reduction in culicine blood-feeding was similar on both unweathered and 3 months weathered tarpaulins. On the 6 months weathered sheeting there was a significant decline in treatment induced reduction in blood-feeding to less than half that seen on the 3 months weathered sheeting. Anopheline blood-feeding was higher than culicine blood-feeding. Treatment induced reduction in anopheline feeding was lower on the insecticide-treated sheeting after weathering. The 6 months weathered sheeting, however, still resulted in a significant reduction in blood-feeding of both culicine and anophelines.

The pre-treated tarpaulins weathered for a 13.5 months period were examined by insectary bioassay (Table 4-9). The pre-treated sheeting is white on one side and black on the other, two tarpaulins were erected for weathering and testing, one with the black side facing outermost and one with the white side.

At baseline (unweathered) the sprayed sheeting resulted in poor mortality for a freshly treated material, with less than 80% mortality after a 24 hour holding period. This sheeting gave lower mortality than the pieces tested in the previous study when the SC formulation (used here) was compared to the WP formulation, although confidence intervals of the two results do overlap. In contrast, mosquitoes exposed to the pre-treated (unweathered) sheeting were all dead after the 24h holding period.

After 3 months the sprayed sheeting had declined considerably in efficacy with only 29 of 67 mosquitoes dead 24 hours after the exposure period. There was no further significant decline after this period. The rate of declining efficacy in the first three month period was similar to that seen in the previous study.

The pre-treated sheeting showed a much slower initial rate of decline, with mosquito mortality greater than 90% after 3 months of weathering. Over the 3 to 6 month period of weathering a considerable loss of efficacy is apparent though after 6 months there is little further change. After 13.5 months mortality in mosquitoes exposed to the pre-treated sheeting is approximately 75%.

The persistence of bio-available insecticide does not appear to depend on whether the pre-treated sheets were erected with the black side or the white side outermost. The trend in mortality over the weathering period was similar on both types of tarpaulin.

Table 4-7. 24 hour mortality of mosquitoes on pre-treated or untreated sheeting over a period of weathering, in overnight platform trails with human sleepers in August and October 2002.

		Culicines				Anophelines ⁵		
	No. of nights	No. of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality		No. of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality
STUDY 3a - Human sleepers, Aug-02	Pre-treated sheeting, unweathered	16	585.7 (215.6)	25.9% ^a (25.1 - 26.8)	18.5%	61.1 (40.2)	46.4% ^a (43.3 - 49.5)	33.8%
	Pre-treated sheeting, 3 m weathered	16	635.1 (221.9)	15.7% ^b (15.0 - 16.4)	7.3%	76.0 (37.1)	30.5% ^b (28.0 - 33.2)	14.2%
	Pre-treated sheeting, 6 m weathered	16	629.6 (140.5)	13.7% ^c (13.1 - 14.4)	5.1%	77.2 (53.2)	25.9% ^c (23.5 - 28.4)	8.5%
	Untreated sheeting	16	648.5 (217.2)	9.1% ^d (8.6 - 9.7)	-	77.1 (50.1)	19.0% ^d (16.9 - 21.3)	-
STUDY 3b - Calf as bait, Oct-02	Pre-treated sheeting, unweathered	15	433.9 (106.1)	32.3% ^a (31.1 - 33.4)	28.1%	109.3 (46.2)	65.0% ^a (62.6 - 67.3)	62.8%
	Pre-treated sheeting, 3 m weathered	15	463.8 (128.1)	19.1% ^b (18.2 - 20.0)	14.1%	100.0 (32.7)	34.6% ^b (32.2 - 37.0)	30.4%
	Pre-treated sheeting, 6 m weathered	15	411.0 (84.6)	15.6% ^c (14.7 - 16.5)	10.4%	157.5 (70.8)	21.1% ^c (19.5 - 22.8)	16.1%
	Deltamethrin SC sprayed sheeting, 3 m weathered	15	433.7 (82.6)	21.6% ^d (20.6 - 22.6)	16.8%	127.7 (44.0)	23.1% ^c (21.2 - 25.0)	18.2%
	Untreated sheeting	15	374.3 (63.4)	5.8% ^e (5.2 - 6.4)	-	120.2 (45.1)	6.0% ^d (5.0 - 7.0)	-

Notes:

1. Percentage mortality and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model.
2. Within each study part and genera, rows not sharing a superscript letter differ significantly ($P < 0.05$) in the blocked logistic regression model.
3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used, wild caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included merely to indicate the size of the denominator for calculation of percentages.
4. Due to low numbers of individual species all anophelines have been grouped.

Table 4-8. Blood-feeding of mosquitoes on pre-treated or untreated sheeting over a period of weathering, in overnight platform trials with human sleepers in August and with calves in October 2002.

		Culicines			Anophelines ⁵			
		No. of nights	No. of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood- feeding	No. of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood- feeding
STUDY 3a - Human sleepers, Aug-02	Pre-treated sheeting, unweathered	16	585.7 (215.6)	4.5% ^a (4.1 - 4.9)	42.3%	61.1 (40.2)	20.4% ^a (18.0 - 23.0)	11.7% n.s
	Pre-treated sheeting, 3 m weathered	16	635.1 (221.9)	5.1% ^b (4.7 - 5.5)	34.6%	76.0 (37.1)	17.3% ^b (15.3 - 19.6)	25.1% n.s
	Pre-treated sheeting, 6 m weathered	16	629.6 (140.5)	6.4% ^c (5.9 - 6.9)	17.9%	77.2 (53.2)	20.9% ^a (18.7 - 23.2)	9.5% n.s
	Untreated sheeting	16	648.5 (217.2)	7.8% ^d (7.3 - 8.4)	-	77.1 (50.1)	23.1% ^a (20.8 - 25.5)	-
STUDY 3b - Calf as bait, Oct-02	Pre-treated sheeting, unweathered	15	433.9 (106.1)	1.4% ^a (1.1 - 1.7)	81.1%	109.3 (46.2)	23.4% ^a (21.4 - 25.5)	38.3%
	Pre-treated sheeting, 3 m weathered	15	463.8 (128.1)	1.6% ^a (1.3 - 1.9)	78.4%	100.0 (32.7)	31.3% ^b (29.0 - 33.7)	17.4%
	Pre-treated sheeting, 6 m weathered	15	411.0 (84.6)	5.2% ^b (4.7 - 5.8)	29.7%	157.5 (70.8)	28.6% ^b (26.8 - 30.4)	24.5%
	Deltamethrin SC sprayed sheeting, 3 m weathered	15	433.7 (82.6)	3.6% ^c (3.2 - 4.1)	51.4%	127.7 (44.0)	28.8% ^b (26.8 - 30.8)	24.0%
	Untreated sheeting	15	374.3 (63.4)	7.4% ^d (6.7 - 8.1)	-	120.2 (45.1)	37.9% ^c (35.7 - 40.1)	-

Notes:

1. Percentage blood-fed and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model.
2. Within each study part and genera, rows not sharing a superscript letter differ significantly ($P < 0.05$) in the blocked logistic regression model.
3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used, wild caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included merely to indicate the size of the denominator for calculation of percentages.
4. Due to low numbers of individual species all anophelines have been grouped.

Table 4-9. Fixed-time insectary bioassays on prototype pre-treated sheeting and deltamethrin sprayed sheeting weathered for varying lengths of time: mosquito mortality after 3 min exposure.

	Unweathered	3 months weathering	6 months weathering	8.5 months weathering	13.5 months weathering
	% mortality, 95% CI				
Deltamethrin SC sprayed sheeting	77.8% (65.9 - 86.4)	43.3% (32.0 - 55.3)	39.1% (28.0 - 51.4)	34.3% (24.0 - 46.4)	42.2% (30.8 - 54.5)
Pre-treated sheeting weathered white side out	100% (100 - 100)	96.7% (87.8 - 99.2)	59.6% (46.5 - 71.5)	79.7% (68.1 - 87.8)	76.9% (65.2 - 85.6)
Pre-treated sheeting weathered black side out	100% (100 - 100)	90.3% (80.1 - 95.6)	65.2% (53.0 - 75.6)	72.1% (60.3 - 81.4)	75.4% (63.1 - 84.6)

Notes:

1. Mortality on untreated sheeting tested as a negative control was never greater than 5%.
2. Percentage mortality and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model.
3. All materials were tested on the inner surface.
4. Bioassays of all items were performed 13.5 months after the start of the trial: *i.e.* items weathered for 0, 3, 6, and 8.5 months were stored after that period of weathering in aluminium foil under refrigeration, bioassays then took place simultaneously at the 13.5 month point to allow for comparison between weathering periods yet avoiding inter-batch variation.
5. Six replicate tests of batches of 10 or 11 mosquitoes were performed on each sheeting type for each period of weathering: 3 pieces of sheeting, cut from the same side of the shelter, were each tested 2 times.

Study 4: An evaluation of the efficacy of Zerofly pre-treated plastic sheeting.

Mosquito mortality when Zerofly sheeting was used was twice that seen when men slept under untreated shelters, both for culicines ($P < 0.001$) and anophelines ($P < 0.001$). Blood-feeding, in this August trial using human sleepers, was not affected by the type of shelter; the proportion blood-feeding was similar on Zerofly and on the untreated sheeting for both culicines ($P = 0.403$) and anophelines ($P = 0.121$).

Table 4-10. 24 hour mortality of mosquitoes on Zerofly or untreated sheeting in overnight platform trials with human sleepers in August 2003.

	No. of nights	Culicines			Anophelines ⁵		
		No. of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality	No. of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality
Zerofly	15	463.5 (146.2)	32.1% ^a (31.0 - 33.2)	19.5%	39.9 (12.7)	68.3% ^a (64.4 - 71.9)	48.8%
Untreated sheeting	15	429.4 (97.3)	15.6% ^b (14.7 - 16.5)	-	42.2 (18.3)	38.1% ^b (34.4 - 41.9)	-

Table 4-11. Blood-feeding of mosquitoes on Zerofly or untreated sheeting in overnight platform trials with human sleepers in August 2003.

	No. of nights	Culicines			Anophelines ⁵		
		No. of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood-feeding	No. of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood-feeding
Zerofly	15	463.5 (146.2)	5.6% ^a (5.1 - 6.2)	No reduction	39.9 (12.7)	11.5% ^a (9.2 - 14.3)	No reduction
Untreated sheeting	15	429.4 (97.3)	5.3% ^b (4.8 - 5.9)	-	42.2 (18.3)	8.8% ^b (6.9 - 11.3)	-

Notes to Tables 4-10 and 4-11:

1. Percentage dead, blood-fed and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model.
2. Within each genera, rows not sharing a superscript letter differ significantly ($P < 0.05$) in the blocked logistic regression model.
3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used; wild caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included merely to indicate the size of the denominator for calculation of percentages.
4. Due to low numbers of individual species all anophelines have been grouped.

Study 5: An evaluation of the insecticidal persistence of Zerofly pre-treated sheeting.

The insecticidal activity of the commercially available pre-treated sheeting product, Zerofly, appeared to be less persistent than the prototype product (Table 4-12).

In fixed-time exposure bioassays the percentage of mosquitoes knocked-down 1 hour after exposure remained high throughout the 10 month weathering period. However, recovery during the 24 hour holding period was seen throughout; with considerably more recovery the longer the sheeting was weathered (Table 4-12). The percentage of mosquitoes killed fell by 69.4% after just 3 months of weathering and continued to decline with half as many mosquitoes again, killed by the sheeting weathered for 6 months. The sheeting weathered for 10 months performed no differently from the 6 months weathered sheeting in these tests.

There was little difference in mortality or knockdown between those mosquitoes exposed for 3 minutes and those exposed for 10 minutes. Some mosquitoes would have been knocked down within the period of exposure itself. These tests were carried out in WHO plastic bioassay cones, any mosquitoes knocked-down within the exposure period would therefore fall to the base of the testing area where the cone meets the sheeting (which was pinned vertically to a pin-board). Many of these mosquitoes would therefore no longer be in contact with the insecticide once they have been knocked-down. In this case it was not surprising that there was little difference in mortality between the mosquitoes exposed for 3 minutes and those exposed for 10 minutes. What little difference was seen was as expected, with those mosquitoes exposed for the longer period more likely to be dead after 24 hours.

In the overnight assays the unweathered Zerofly resulted in a high level of treatment induced mortality in both culicines and anophelines (Table 4-13). These tests tell a similar story to the insectary bioassays with regard to the persistence of the insecticide treatment. Treatment induced mortality on the 10-months weathered sheeting was far lower than that on the unweathered sheeting. This held true both for culicine and anopheline mortality.

There was no evidence for a change in culicine or anopheline blood-feeding rate by any of the insecticide treatments whether weathered or unweathered (Table 4-14).

Table 4-12. Fixed-time exposure and median time to knock-down insectary bioassays on Zerofly plastic sheeting over a period of weathering: 1 h knockdown and 24 h mortality after 3 minutes or 10 minutes of exposure and median time to knockdown (MTKD).

	Unweathered	3 months weathering	6 months weathering	10 months weathering
	% mortality, (95% CI)			
3 minute exposure	80.0 (72.8 - 85.6)	24.5 (18.1 - 32.2)	12.1 (7.7 - 18.4)	11.3 (7.2 - 17.5)
10 minute exposure	88.1 (81.7 - 92.5)	26.2 (19.7 - 34.0)	20.1 (14.5 - 27.2)	17.6 (12.2 - 24.6)
	% knockdown, (95% CI)			
3 minute exposure	94.7 (89.7 - 97.3)	87.4 (80.9 - 91.9)	98.0 (93.9 - 99.3)	90.7 (84.9 - 94.4)
10 minute exposure	97.9 (93.7 - 99.3)	82.8 (75.7 - 88.1)	96.8 (92.4 - 98.6)	91.9 (86.3 - 95.3)
	MTKD, seconds (95% CI)			
Continual exposure until knockdown	541.5 (464.7 - 618.3)	835.5 (782.7 - 888.3)	897.5 (849.8 - 945.3)	1138.9 (1033.5 - 1244.3)

Notes:

1. Mortality on untreated sheeting tested as a negative control was never greater than 5%. In MTKD tests no mosquitoes exposed to untreated sheeting as a negative control were knocked-down after a 30-minute (1800 seconds) test period.
2. Percentages knocked-down, dead and their 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model.
3. The surface of the Zerofly sheeting facing inwards during weathering was tested.
4. Bioassays of all items were performed 10 months after the start of the trial: *i.e.* pieces weathered for 0, 3 and 6 months were stored after that period of weathering in aluminium foil under refrigeration, bioassays then took place simultaneously at the 10 month point to allow for comparison between weathering periods yet avoiding inter-batch variation.
5. Fifteen replicate tests were performed on each sheeting type for each period of weathering: 3 pieces of sheeting, cut from the same side of the shelter, were each tested 5 times.
6. For median time to knockdown tests each replicate consisted of 11 mosquitoes. The number of mosquitoes tested in fixed-time exposure bioassays are shown in the table.

Table 4-13. 24 hour mortality of mosquitoes on Zerofly sheeting unweathered or weathered for 10 months and on untreated sheeting, in overnight platform tests with calves as bait in November 2003

	No. of nights	Culicines			Anophelines ⁵		
		No. of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality	No. of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality
Zerofly Unweathered	7	199.4 (17.7)	32.4% ^a (30.0 – 34.9)	31.0%	33.6 (9.6)	47.7% ^a (41.3 – 54.0)	45.1%
Zerofly, 10 m weathered	7	220.3 (18.1)	7.3% ^b (6.1 – 8.7)	5.4%	41.6 (10.4)	17.9% ^b (13.9 – 22.7)	13.8%
Untreated sheeting	7	240.3 (15.7)	2.0% ^c (1.4 – 2.7)	-	29.7 (6.6)	4.8% ^c (2.6 – 8.7)	-

Table 4-14. Blood-feeding of mosquitoes on Zerofly unweathered or weathered for 10 months, and on untreated sheeting, in overnight platform trials with calves as bait in November 2003

	No. of nights	Culicines			Anophelines ⁵		
		No. of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood-feeding	No. of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood-feeding
Zerofly Unweathered	7	199.4 (17.7)	3.2% ^a (2.4 – 4.3)	No reduction	33.6 (9.6)	32.8% ^a (27.1 – 39.0)	No reduction
Zerofly, 10 m weathered	7	220.3 (18.1)	6.4% ^b (5.2 – 7.7)	No reduction	41.6 (10.4)	30.6% ^a (25.6 – 36.1)	No reduction
Untreated sheeting	7	240.3 (15.7)	3.1% ^a (2.4 – 4.0)	-	29.7 (6.6)	30.3% ^a (24.4 – 36.9)	-

Notes to Tables 4-13 and 4-14:

1. Percentage mortality, blood-fed and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model.
2. Within each genera, rows not sharing a superscript letter differ significantly ($P < 0.05$) in the blocked logistic regression model.
3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used; wild caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included merely to indicate the size of the denominator for calculation of percentages.
4. Due to low numbers of individual species all anophelines have been grouped.

Study 6: Musca domestica mortality and resting behaviour on exposure in day-time platforms assays to insecticide-treated sheeting unweathered and weathered for 6 months, and untreated sheeting.

In the first round of testing house flies were exposed for 4 hours in outdoor enclosed platforms to either unweathered pre-treated plastic sheeting or untreated sheeting, to determine whether the treated tarpaulins would cause mortality in free-flying houseflies (Table 4-15). In these tests a very high level of treatment induced mortality was seen; almost 90% of the flies exposed to the treated sheeting were dead after the 24 hour holding period (compared to about 40% for untreated sheeting) (Table 4-15). This high treatment induced mortality was seen again in the second round of tests, which took place the following year, where flies were exposed to the treated shelters for only 1 hour, with 97% of the flies killed on the treated sheeting compared to 15% on the untreated sheeting (Table 4-16). In this second round of tests a pre-treated tarpaulin that had been weathered for 6 months was included. On this sheeting fly mortality was less than half that seen on the unweathered pre-treated sheeting but still resulted in treatment induced mortality (35.2%).

In the second round of tests house fly resting site behaviour was also recorded. At each time point the resting sites of all flies were recorded. Flies were observed to rest least often on the unweathered treated plastic sheeting, more often on the treated sheeting that had been weathered for 6 months, and most often on the untreated sheeting. The number of times flies were observed resting within the shelter, on the floor or bowls of sugar, were significantly, but only marginally, different on each type of shelter with no clear trend seen. Flies may not, therefore, be repelled from resting on the sheeting to resting on the occupants or food items within a shelter. Rather, the flies may be repelled away from the shelter itself. The number of times flies rested outside of the shelter, on the trap net enclosing the platform, in the box providing alternate shade, or the floor, was greatest when the unweathered treated shelter was tested, and least when the untreated shelter was tested, with an intermediate result for the 6 months weathered sheeting.

The results are interesting in that relatively few instances of flies resting on the sheeting were recorded, despite the fact that very high mortality was seen when the treated shelters were used. It may be that a very short exposure gives a lethal dose of insecticide. However, no contact bioassays could be carried out at this time due to limited facilities and expertise for house fly rearing and handling.

Table 4-15. Mortality of *M. domestica* 24 hours after 4 hours of exposure to unweathered prototype pre-treated plastic sheeting or untreated sheeting on a covered platform within which the flies were free-flying.

	Number of replicate tests	Number of <i>M. domestica</i> per test Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality
Prototype pre-treated sheeting	10	271.3 (62.2)	89.9% ^a (88.7 - 91.0)	83.7%
Untreated sheeting	10	109.0 (43.6)	38.1% ^b (35.2 - 41)	-

Table 4-16. Mortality of *M. domestica* 24 hours after 1 hour of exposure to unweathered Zerofly sheeting, the prototype pre-treated sheeting weathered for 6 months or untreated sheeting, on a covered platform within which the flies were free-flying.

	Number of replicate tests	Number of <i>M. domestica</i> per test Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality
Unweathered Zerofly sheeting	6	227.3 (157.0)	97.4% ^a (96.4 - 98.2)	96.9%
Prototype pre-treated sheeting weathered for 6 months	4	264.3 (82.3)	44.4% ^b (41.4 - 47.4)	34.7%
Untreated sheeting	5	169.6 (132.1)	14.9% ^c (12.6 - 17.4)	-

Notes to Tables 4-15 and 4-16:

1. Percentage mortality and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model.
2. Within each table, rows not sharing a superscript letter differ significantly ($P < 0.05$) in the blocked logistic regression model.

Table 4-17. Resting sites of free-flying *M. domestica* exposed to treated or untreated plastic sheeting, weathered or unweathered within an enclosed platform.

	Number of replicate tests	number of resting instances recorded Mean (SD) ^{1,2}	% of resting instances occurring on the sheeting ^{3,4a} Mean (95% CI)	% of resting instances occurring within the shelter ^{3,4b} Mean (95% CI)	% of resting instances occurring outside the shelter ^{3,4c} Mean (95% CI)
Zerofly sheeting	6	1837.8 (1210.9)	3.0% ^a (2.7 - 3.3)	13.8% ^a (13.2 - 14.5)	83.2% ^a (82.5 - 83.9)
Prototype pre-treated sheeting weathered for 6 months	4	2566.3 (533.0)	8.3% ^b (7.7 - 8.8)	16.2% ^b (15.5 - 16.9)	75.5% ^b (74.7 - 76.4)
Untreated sheeting	5	2016.0 (806.5)	15.9% ^c (15.2 - 16.7)	14.9% ^c (14.2 - 15.6)	69.1% ^c (68.2 - 70.0)

Notes:

1. Instances of flies resting were recorded at the following time points after flies were released on the platform (at 0 minutes): 1, 2, 3, 4, 5, 10, 20, 40, 60 minutes.
2. Possible resting sites were: outside surface of the sheeting, inside surface of the sheeting, floor within the shelter, floor outside of the shelter, trap-net enclosing the platform, bowl of sugar within the shelter, box providing alternate shade outside the shelter.
3. The total of all instances of resting recorded over all the time points is used as the denominator for analysis, despite the fact that some of the same flies will be recorded at each time point.
4. The % of instances of resting recording as being a) on the sheeting; b) within the shelter (on the floor or bowl of sugar), or c) outside the shelter (on the floor, on the trap net or in the box provided for alternate shade) are used as the numerator for analysis, despite the fact that the same flies will be recorded at each time point.

4.4 Discussion

4.4.1 *The effect of insecticide-treated sheeting on mosquito mortality*

Seven datasets are available to examine mosquito mortality in the presence of unweathered insecticide-treated sheeting, compared with that seen on untreated sheeting (Tables 4-1, 4-3, 4-7, 4-10 and 4-13 as well as those data from the first exploratory study presented in Chapter 3, Table 3-2). Insecticide-treated sheeting increases both culicine and anopheline mortality in all the overnight platform studies carried out. For culicines this effect ranged from a doubling to a 16-fold increase in mortality and for anophelines it ranged from a 1.5-fold increase to a 12-fold increase.

It is likely that environmental factors influenced the magnitude of the effect on mosquito mortality observed in these studies. Table 4-18 shows the culicine and anopheline mortality on untreated sheeting occurring in these studies with the month in which the study took place shown. It appears that natural mosquito mortality is higher in the warmer months than in the tail end of the mosquito season, when perhaps the hardier mosquitoes make up a greater proportion of the population.

Table 4-18. Mosquito mortality on untreated sheeting by month of test

Month	% culicine mortality on untreated sheeting	% anopheline mortality on untreated sheeting
August	9.1; 10.7; 15.6	19.0; 21.5; 38.1
October	5.8 ; 5.8*	6.0* ; 11.3
November	2.0* ; 5.0	4.5 ; 4.8*

*these studies used calves as bait to attract more mosquitoes at the tail end of the mosquito season, the other studies used human sleepers.

In contrast treatment induced mortality is generally lower in the warmer months (Table 4-19). This trend is clearer for culicines than for anophelines, further investigation would be needed to determine if this is a true trend.

Table 4-19. Mosquito mortality on treated sheeting (pre-treated or sprayed on both sides with deltamethrin SC) without weathering, by month of test

Month	% culicines treatment induced mortality	% anophelines treatment induced mortality
August	11.3 ; 18.5 ; 19.5	17.1 ; 33.8 ; 48.8
October	17.0 ; 28.1*	22.9 ; 62.8*
November	31.0* ; 78.0	45.1* ; 98.5

*these studies used calves as bait to attract more mosquitoes at the tail end of the mosquito season, the other studies used human sleepers.

There are possible reasons that such a trend may occur. During the cooler nights mosquitoes may be more inclined to approach the shelters (and therefore be more likely to pick up a lethal dose of insecticide) either to seek warmth or because the warmth of the men or cow within is more apparent, making host seeking easier (though, see section 4.3 below). Alternatively higher mortality in the cooler months may be a result of the effect of temperature on pyrethroids, these products are more toxic at lower temperatures, with some studies showing a clear threshold at 30°C after which mortality is lower. However it is difficult to differentiate the impact of temperature on pyrethroid action at the target site level from the impact of mosquito behaviour.

The very high anopheline and culicine mortalities observed with unweathered pre-treated sheeting in the study presented in the previous chapter, which took place in November at the cool tail-end of the mosquito season, were not replicated in the later trials. Month of test alone is not enough to explain this difference. Nightly temperature readings were not collected throughout these trials but it may be that the tail end of the mosquito in 2001 was cooler or less humid than subsequent years, or vice versa.

In the majority of studies (5 out of 7 for culicine mortality and anopheline mortality the treatment induced mortality was between 17 and 49%). It is likely that induced mortality will usually be in this range although environmental conditions and other host or vector factors may lead to increases or reductions in this effect. It is important that trials of this product take place in other environmental settings. The first trial to take place in a distinct setting, in West Africa, using permethrin treated ITPS showed ~55% induced mortality on the ITPS shelters (Rowland, Pers. Comm.).

The sheeting sprayed with insecticide resulted in similar level of mosquito mortality to the pre-treated shelters, prior to any weathering. Before pre-treated sheeting becomes widely used insecticide spray campaigns in camps using plastic sheeting are likely to continue where the logistics allow.

The results study that examined the effects of sheeting treated on different surfaces suggested that spraying only the inside of the sheeting will not be the most efficacious intervention. It may be that ITPS does not act in the same way as IRS. Shelters made of plastic tarpaulins are rather more open to vectors entering and exiting than houses. It is likely that the vectors' behaviour in such shelters differs markedly from that in houses, in terms of resting before entering and behaviour after feeding. For example, more exophagic or exophilic vectors are likely to feed on

people sleeping in fairly open tarpaulin shelters than they are in houses and these mosquitoes may behave differently after feeding. However, these results should be interpreted with caution, the very low blood feeding seen suggests that the normal host seeking behaviour of the mosquitoes may have been interrupted, as discussed further below, studies with slightly modified methodology would be useful to confirm these interpretations.

4.4.2 *The effect of treated sheeting on blood-feeding*

Seven datasets are available to examine mosquito blood-feeding in the presence of unweathered insecticide-treated sheeting, compared with that seen on untreated sheeting (Tables 4-2, 4-4, 4-8, 4-11 and 4-14 as well as those data from the first exploratory study presented in Chapter 3, Table 3-3). In some studies no reduction in blood-feeding was observed when men (or calves) slept in treated shelters, whereas in others studies some reduction was apparent.

There is no apparent trend by month in the proportion of mosquitoes successfully taking a blood-meal when untreated shelters are present (Table 4-20). This would suggest that a theory of host seeking being easier as a result of a greater difference between the host temperature and the ambient temperature in cooler months (as mentioned above) is unlikely.

For culicines, in 3 of the 7 datasets no reduction in feeding was observed, but in 4 sets treatment induced reduction in blood-feeding was between 29% and 43%. For anophelines, no reduction of blood-feeding was seen in 4 of the 7 datasets, whilst in the other 3 studies a treatment induced reduction in blood-feeding between 11% and 54% was observed.

The failure to demonstrate consistent protection from biting across different studies with treated tarpaulins means that prevention or control of malaria will mostly depend upon generating high mortality among the vector population ("mass effect") rather than direct protection from biting. Other recent studies in West Africa evaluating permethrin ITPS also showed only a small reduction in blood-feeding; 75% of the anthropophilic vectors were still able to feed on men sleeping under the treated sheeting compared to ~95% when untreated sheeting was used (Rowland, Pers Comm.). The prospect for disease control may remain high because coverage in new refugee camps would approach 100% as a result of free distribution of tarpaulins on registering of refugees. However, the further studies recommended above, to determine the magnitude of the effect of ITPS on mosquito mortality in other environmental settings, would be useful to support this.

Table 4-20. Mosquito blood-feeding on untreated sheeting by month of test

Month	% culicines blood-fed in presence of untreated sheeting	% anophelines blood-fed in presence of untreated sheeting
August	5.3 ; 7.8 ; 10.6	8.8 ; 23.1 ; 32.5
October	7.2 ; 7.4*	5.2 ; 37.9*
November	3 ; 3.1*	9 ; 30.3*

*these studies used calves as bait to attract more mosquitoes at the tail end of the mosquito season, the other studies used human sleepers.

Overall the level of blood-feeding seen in these study are lower than those observed in previous studies at the same study site where an “open net” testing methodology has been used (Hewitt *et al.*, 1995). Hewitt and colleagues examined the effect of permethrin-sprayed tents on mosquito populations, both the method used in the present studies (“closed-net”), and an alternate “open-net” method were compared. In the open-net method the platforms were arranged as in the present studies but the trap-net was raised about 30cm from the ground to allow host seeking mosquitoes to enter naturally. One hour before sunrise the net was then closed to prevent mosquitoes from leaving. In the open-net method the majority of dead mosquitoes were of those that had succeeded in feeding, in the closed-net methods, as in the studies presented here, the majority of dead mosquitoes were unfed. The open-net method has the advantage of not interrupting the host-seeking behaviour pattern, the closed-net method, where mosquitoes are attracted to the platform and then manoeuvred with mouth aspirators to the inside of the net may result in disorientation of the mosquitoes in less blood-feeding behaviour. This, rather than an assumption that mosquitoes rest on the outside surface of a shelter picking up an insecticide dose before reaching the host, may account for the lower blood-feeding seen.

The reason that this closed-net method was used here is that the open net method has different disadvantages, the number of mosquitoes escaping from the net during the night is unknown, the proportion of those escaping that have picked up a lethal dose of insecticide is unknown, the proportion of those escaping that have succeeded in taking a blood meal is unknown.

However, it may be that trials including both methods, or a further modification of the testing method would give a truer picture of the effect of these shelters on host seeking mosquitoes.

4.4.3 Resistance to weathering

An earlier evaluation of permethrin-sprayed canvas tents in Pakistan showed a decay of residue within a few months of spraying the inner surfaces on single-sheeted tents (Bouma *et al.*, 1996b). In the studies presented here plastic sheeting

sprayed with deltamethrin is also seen to lose efficacy over a 3 month period (with the suspension concentrate formulation losing efficacy less rapidly than the wettable powder formulation). The clear decline in efficacy of the SC formulation apparent in insectary bioassay (Tables 4-5, 4-6 and 4-9) did not always translate to a poorer performance after weathering in overnight platform trials: the performance of the 3-month weathered sheeting (in the summer with human sleepers) was equal to that of unweathered sheeting (Tables 4-3 and 4-4); however, later in the season, when tested with calves as bait, the 3 months weathered sheeting performed considerably worse than the unweathered pre-treated sheeting (Tables 4-7 and 4-8).

The pre-treated sheeting also showed a decline in efficacy after weathering. However, the pre-treated sheeting was much more resistant to weathering than the sprayed sheeting tested alongside the prototype product. Whilst the bio-available insecticide on the prototype product appeared to decline initially and then stabilise over a 13 month period of weathering, for Zerofly the decline continued over the 10 month period of weathering. This declining efficacy apparent in bioassays translates to a poorer performance after weathering in overnight trials, where there was a clear trend of declining mortality with weathering for both the prototype sheeting and the Zerofly.

While it is important that pre-treated sheeting is able to withstand weathering, the declining efficacy observed over several months is not a great blow to its usefulness if deployed in refugee camps. The acute stage of the emergency, when mortality rates are highest, is the period when conventional malaria control is often thwarted by logistic and security constraints (Rowland and Nosten, 2001). Plastic tarpaulins are distributed during that initial influx of refugees. A few months later, as the camp becomes better established, refugees usually erect their own homes using locally available materials. Plastic tarpaulins may be retained as useful waterproofing for roofs or walls but may also be sold on. The insecticidal activity of the tarpaulin need only last as long as IDPs and refugees are using the tarpaulins as their main shelter. Once the camp moves into the post-emergency stage, conventional methods of malaria control (e.g. ITN, IRS) are more easily applied and may be needed to provide an effective alternative vector control strategy once the plastic sheeting is no longer useful.

Two major factors may influence the effectiveness of the plastic sheeting in use in refugee camps.

- i) The local environmental conditions: in these overnight studies the magnitude of the effect on mosquito blood-feeding and mortality appeared to change over the transmission season. Temperature, humidity and local vector behaviour may affect the level of “mass-effect” or protection seen in these settings. The level of UV radiation, higher nearer the equator than in our trial may cause more rapid degradation of insecticide.
- ii) The way in which the shelter is erected: The promising results achieved here in overnight platform trials were with sheeting erected in a ridge-pole tent design. It is not clear how far results using such a design can be extrapolated to shelters constructed with the same material but used in other ways. When tarpaulins are distributed to arriving refugees for shelter, methods of support and shelter design may vary. Even when shelters are built to a standard design initially (which may well not correspond to the ridge-pole design) they are likely to quickly be modified by the inhabitants.

4.4.5 Effect of pre-treated sheeting on Musca domestica.

The high mortality amongst house flies exposed to treated sheeting in these free-flying trials suggests that this tool may have additional benefits than malaria control. Diarrhoeal diseases are often the most important cause of mortality in refugee camps (Toole and Waldman, 1997). The significant role of houseflies in the transmission of some diarrhoeal diseases (Cohen *et al.*, 1991; Chavasse *et al.*, 1999) indicates that the potential of pyrethroid treated sheeting to reduce housefly numbers could be beneficial. The results of the tests recording the resting behaviour of flies when exposed to treated and untreated sheeting is encouraging, a treated shelter which prompted flies to rest on the inhabitants of the shelter or their food products rather than the insecticidal sheeting would be disadvantageous, it seems likely that this would not be the case. Following up these platform assays with a disease control trial of ITPS for diarrhoeal disease episodes would be an appropriate next step.

Leishmaniasis is another vector borne disease that can be controlled by residual spraying (Pandya, 1983; Vioukov 1987; Reyburn *et al.*, 2000) and therefore potentially controllable by ITPS. Insecticide-treated sheeting has potential as a wider public health tool against a variety of vector borne diseases in refugee camps, alongside its promise as a tool against malaria in the problematic acute phase.

Chapter 5. An entomological evaluation of tents pre-treated with insecticide

5.1 Introduction

Spraying tents with insecticide was originally used as a malaria control tool for nomadic peoples (Motobar *et al.*, 1974). The early work with DDT and dieldrin had little success owing to the poor adhesion to fabrics of the formulations then available (wetable powder) (Motobar *et al.*, 1974; Bouma *et al.*, 1996a). Pyrethroid insecticides in suspension concentrate or micro-encapsulated formulations show better adhesion and residual efficacy and are more suitable for treatment of textiles. This has enabled successful treatment of tents in recent years.

Entomological evaluations have shown that canvas tents sprayed with pyrethroids can reduce mosquito feeding and cause high knock-down and mortality (Schreck, 1991; Heal *et al.*, 1995). Tents sprayed with permethrin and deltamethrin reduced *Anopheles stephensi* biting by about 40% and resulted in 75% mortality among blood-fed mosquitoes (Hewitt *et al.*, 1995). The efficacy of permethrin-treated tents for controlling malaria was demonstrated when this strategy was used to control an epidemic of falciparum malaria in a population of nomadic Afghan refugees in Pakistan (Bouma *et al.*, 1996a).

The evidence, both entomological and epidemiological, for pyrethroid-sprayed tents as an appropriate tool for malaria control in refugee camps has led to this technology being included in several refugee health care manuals (Thomson, 1995; MSF, 1997; UNHCR, 1999). In recent years they have been implemented as a control method in numerous refugee and IDP settings (e.g. Sierra Leone and Mozambique).

It is at the early acute stage of an emergency, when refugee camps are first established that poor sanitation, malnutrition and mortality due to disease are at their worst and the environment is particularly suitable for transmission of vector-borne diseases. Conventional responses to malaria control may be difficult due to insecurity, inaccessibility and inadequate inter-agency co-ordination. Organisation and implementation of insecticide spray campaigns is logistically demanding and may not be feasible at this stage. Logistical efforts are more likely to be focused on the delivery of emergency food, medicine, clean water, blankets and shelter. If the shelter materials that are distributed during camp construction were pre-treated, a potentially effective vector control tool could be delivered with no extra demand on

logistical resources. Pre-treated polyethylene laminated tarpaulins (Zero-fly™) and tents (both Vestergaard Frandsen), have already been demonstrated to cause mortality and reduce blood-feeding of malaria vectors (Graham *et al.*, 2002b; Chapters 3 and 4). Untreated plastic tarpaulins, similar to those tested, are frequently distributed to refugees. As an alternative or an addition to plastic tarpaulins, canvas tents are often distributed. Tents made completely of plastic sheeting are unlikely to be suitable as shelter materials as the conditions inside the shelter are considerably hotter and more humid than the canvas tents usually distributed in a refugee camp. To address these issues a canvas tent incorporating pre-treated deltamethrin-impregnated polyethylene fibres has been developed.

An entomological evaluation of these tents in Afghan refugee camps in Pakistan is presented here.

5.2 Materials and methods

5.2.1 Study location

Insectary bioassays and overnight platform trials with insectary-reared mosquitoes were carried out at the HealthNet International field site in Adizai refugee settlement. Overnight platform trials with wild-caught mosquitoes took place at the HealthNet International entomological field station in Azakhel refugee settlement. Both field stations are described in more detail above (Chapter 2, Section 2.1)

5.2.2 Materials

The tents are made mostly of untreated canvas. Deltamethrin-treated polyethylene threads (of the same material used to make Zero-fly™) are interwoven through the canvas fabric during manufacture. This composite material has a cream and blue striped appearance (Figure 5-1). The tents have doors at both ends; each door is made up of outer canvas door-flaps and inner mosquito mesh door-flaps which can be sealed closed with Velcro. The mosquito mesh door-flaps are made from PermaNet™ polyester netting, which is pre-treated with deltamethrin.

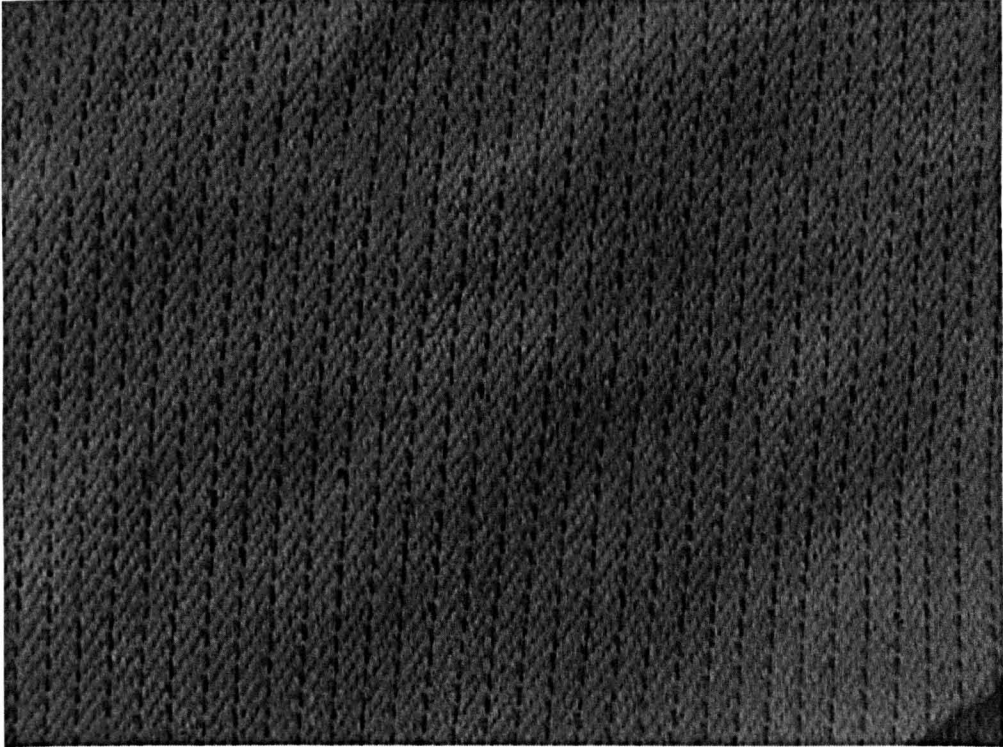


Figure 5-1. Close-up of tent material: untreated canvas (the lighter coloured areas, in reality cream coloured) with deltamethrin-treated polyethylene thread (the thin darker stripes, in reality blue) woven through.

5.2.3 *Contact bioassays*

Fixed-time exposure bioassays for 3 minutes and 10 minutes were performed using the methodology described above (Chapter 2, Section 2.2). These took place over a 3 month period of weathering.

In order to keep the tents intact for overnight platform bioassays insectary bioassays were carried out on the inside surfaces of the tents as they stood in position for the weathering period, rather than cutting samples and taking them to the bioassay room for testing. Plastic bioassay cones were taped to the inside of the tents on four places on the roof, these places were marked to ensure the same spot was tested each month.

5.2.4 *Overnight platform trials with wild-caught mosquitoes*

Overnight platform bioassays were carried out following the method described above (Chapter 2, Section 2.2). Four men slept on bed-rolls on the floor of each tent in local dress (cotton shalwar-chemise), each covered by a woollen blanket. At one end of the tent the doors were securely closed, at the other end the canvas door

flaps were tied open whilst the mesh doors hung loose. The closed end was alternated nightly between the two ends of the tent.

These tests took place during November 2003.

5.2.5 Overnight platform trials with insectary-reared mosquitoes

Overnight tests were carried out with insectary-reared mosquitoes in January and February 2004, after the natural mosquito season had ended. Tents and trap-nets were erected as above (Chapter 2, section 2.2). A cow was tethered within the tents in the place of sleepers. *An. stephensi* and the other local vectors are highly zoophilic and cattle make a suitable alternative host.

Approximately 400 unfed, 5-7 day old, insectary-reared, *An stephensi* of both sexes were released into the trap-nets at dusk, collected at dawn, put in holding cages with access to sugar solution and held in insectary conditions for 24 hours. Mosquitoes were sexed and categorized as blood-fed or unfed and dead or alive. Only female mosquitoes were included in the analysis.

5.2.6 Statistical analysis

Proportional data (mortality and blood-feeding) from the platform trials and the contact bioassays were analysed using blocked logistic regression (STATA 6 software). Comparisons between treatments were made by successively dropping treatments from the overall comparison. This process allows each treatment to be compared with every other. Means and confidence intervals of the constant for each treatment were back-transformed for presentation as follows:

$$x' = \frac{1}{1 + (1/\exp(x))}$$

Where: x' = back-transformed value

x = the value from the logistic regression

5.3 Results

During the overnight platform study with wild caught mosquitoes an average of 11.9 ± 3.3 (\pm standard error) anophelines and 143.8 ± 6.9 culicines were caught per platform per night. The majority of anophelines were *An. subpictus* (7.5 ± 3.2), *An. culicifacies* (1.9 ± 0.4) and *An. stephensi* (1.2 ± 0.3) plus smaller numbers of *An. annularis*, *An. fluviatilis* and *An. pulcherrimus*.

Both the 3 and 10 minute exposure contact bioassays, resulted in 100% knock-down of mosquitoes within one hour of exposure (Table 5-1). Some recovery during the holding period resulted in lower than 100% mortality after 24 hours in both the 3 minute and 10 minute tests (93.2% and 97.4% respectively).

Overnight platform tests with wild-caught host-seeking mosquitoes in the presence of a treated tent resulted in significantly higher mosquito mortality and significantly lower blood-feeding rates than when an untreated tent was tested (Table 5-3). The proportion killed approximately doubled for culicines ($P < 0.001$) and anophelines ($P = 0.001$), whilst blood-feeding was reduced five-fold (culicines $P = 0.001$, anophelines $P < 0.001$).

In overnight tests with insectary-reared *An. stephensi* and calves tethered inside the tents baseline mortality and blood-feeding (*i.e.* on the untreated tent) were higher than in the aforementioned overnight tests with wild-caught mosquitoes and human sleepers (Table 5-2). In the presence of the deltamethrin-treated tent there was a significant increase in mosquito mortality and reduction in blood-feeding. In the presence of the treated net mosquito mortality increased 15-fold from that on the untreated tent ($P < 0.001$) and blood-feeding was reduced to less than half ($P < 0.001$).

After one month of weathering 1 hour knock-down and 24 hour mortality were greater than 95% in both the 3 minute and 10 minute tests (Table 5-1). Some decline in insecticidal effect was seen after two and three months weathering, but after 3 months both knock-down and mortality remained greater than 80% in 3 minute exposure bioassays and greater than 90% in 10-minute exposure bioassays.

Table 5-1. Knock-down and 24 hour mortality in contact bioassays on the inside surface of insecticide-treated tents after weathering.

		Unweathered	1 month weathering	2 months weathering	3 months weathering
Knock-down 1h after exposure	3 minute exposure	100% (100 - 100)	100% (100 - 100)	92.9% (85.2 - 96.8)	81.2% (74.8 - 87.8)
	10 minute exposure	100% (100 - 100)	96.3% (89.3 - 98.8)	85.2% (76.2 - 91.2)	93.0% (88.1 - 96.0)
Mortality 24h after exposure	3 minute exposure	93.2% (84.8 - 97.2)	100% (100 - 100)	63.5% (52.8 - 73.0)	83.0% (76.7 - 87.8)
	10 minute exposure	97.4% (90.2 - 99.3)	97.6% (90.8 - 99.4)	80.7% (71.1 - 87.6)	91.3% (86.0 - 94.7)

Table 5-2. Blood-feeding and mortality in overnight platform tests with insectary-reared *An stephensi* and calves as bait in January and February 2004.

	Number of replicates	Number of mosq. per night Mean(SD) ³	% blood-fed (95% CI)	% 24h mortality (95% CI)
Insecticide-treated canvas tent	14	257 (76)	22.2%** (20.9 - 23.6)	80.9%** (79.5 - 82.1)
Untreated canvas Tent	7	262 (105)	51.1%** (48.8 - 53.4)	5.2%** (4.3 - 6.3)

Notes to Tables 5-1 and 5-2:

1. In Table 5-1 numbers of mosquitoes knocked-down or dead over the total tested are shown with percentages knocked-down or dead and 95% CI.
2. In Table 5-1 six replicate tests with batches of 10 or 11 mosquitoes were performed at each point.
3. Percentage blood-fed, knock-down, mortality and 95% confidence intervals are back-transformed from values calculated by the blocked logistic regression model.
4. Significance levels between results on the treated tent and untreated tent for each genera are indicated with asterix: *=P=0.001 ; **P<0.001 (Table 5-2)
5. The mean number of mosquitoes per treatment per night (Table 5-2) does not provide information about the relative attractiveness of each test item: enclosed platforms were used; wild-caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included for reference purposes only. These means do not differ significantly by t-test.
6. Alongside the contact bioassays presented in Table 5-1 tests were performed on an untreated tent as a control. 24 hour mortality was never more than 5%.

Table 5-3. Blood-feeding and mortality of wild-caught mosquitoes on treated and untreated tents in overnight platform trials with human sleepers in November 2003.

	Number of replicates	Culicines			Anophelines ⁴		
		Number of mosq. per night Mean(SD) ³	% blood-fed (95% CI)	% 24h mortality (95% CI)	Number of mosq. per night Mean(SD) ³	% blood-fed (95% CI)	% 24h mortality (95% CI)
Insecticide-treated canvas tent	9	143 (31)	0.4%* (0.2 – 0.9)	39.4%** (36.7 – 42.1)	7.2 (4.1)	9.2%** (4.2 – 19.1)	50.8%* (38.8 – 62.6)
Untreated canvas Tent	6	145 (22)	2.0%* (1.2 – 3.1)	17.1%** (14.8 – 19.8)	18.8 (18.2)	46.0%** (37.1 – 55.2)	25.7%* (18.5 – 34.5)

Notes:

1. Percentage blood-fed mortality and 95% confidence intervals are back-transformed from values calculated by the blocked logistic regression model.
2. Significance levels between results on the treated tent and untreated tent for each genera are indicated with asterix: *=P=0.001 ; **P<0.001
3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used, wild-caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included for reference purposes only. These means do not differ significantly by t-test.
4. Due to low numbers of individual species all anophelines have been grouped.

5.4 Discussion

The pre-treated canvas-polyethylene tents clearly affect mortality and blood-feeding rates of both wild and insectary-reared malaria vectors. The lower control mortality and higher treatment mortality observed with insectary-reared mosquitoes cannot be confidently assigned to genetic or behavioural consequences of insectary colonization because of possible confounding effects due to the use of cattle as bait and testing during the cooler months of January and February (tests with wild mosquitoes were done during November using humans as bait). Whilst the magnitude of the effect differs between the trials with insectary-reared and with wild-caught mosquitoes, the conclusions to be drawn are the same.

The manufacturer of the tents tested here have combined the technology of the deltamethrin-impregnated polyethylene (from which Zerofly™ are constructed) with the accepted design of a canvas ridge-pole tent. Whilst tents made entirely of plastic sheeting were shown to be effective against malaria vectors on a previous occasion (Graham *et al.*, 2002a; Chapter 3), tents of canvas and polyethylene may be more suitable for use in refugee camps. The superior design of these canvas-polyethylene tents was commented on by the refugee helpers involved in this trial who had also been involved in the trial of plastic tents. The sleepers reported that the canvas tents were cooler and less humid than the plastic tents (no comparative temperature or humidity data were collected during this study).

Further entomological studies are needed to assess the persistence of the insecticide. A canvas tent will be in use for longer than a plastic tarpaulin and it is, therefore, important that the residual life of the insecticide treatment is documented and, if necessary, prolonged by appropriate use of UV filters. Persistence of insecticide that gives > 90% mortality in 10-minute exposure cone bioassay tests after a year of weathering would be an acceptable target. Deltamethrin has shown good persistence when sprayed on the inner surfaces of double-sheeted tents (Hewitt *et al.*, 1995) but persistence after spraying on inner and outer surfaces of single sheeted tents has not been documented. Outdoor weathering of the tent should continue to be monitored, with contact bioassays conducted on a monthly basis and with further overnight trials after a period of several months to one year.

The use of pyrethroid-treated tents is already established as a malaria control intervention. A technology that enables tents to be pre-treated with insecticide during manufacture and be shown to retain insecticidal efficacy for up to one year

would improve the feasibility of malaria control during the acute stage of an emergency.

These findings on 0 to 3 month weathered tents demonstrate that this technology is equivalent to deltamethrin-sprayed canvas tents over this period. If the criteria of adequate insecticidal persistence is met, this technology could be recommended as a good tool for malaria control in refugee camps, without the need for extensive disease control trials.

Chapter 6. A comparison of three pyrethroids for the treatment of top-sheets: analysis of previous entomological and user acceptance studies

6.1 Introduction

Insecticide-treated mosquito nets (ITNs) and other materials serve as a vehicle for efficient delivery of insecticide (usually a fast-acting pyrethroid) for personal and community protection against mosquitoes, sandflies, lice and other biting insects, especially to limit the risks of vector-borne disease transmission. Pyrethroid impregnation of blankets has the potential to provide such protection without needing to deal with the extra financial, logistic and cultural issues raised by the distribution of ITNs, factors which limit the feasibility of ITNs as an option at the early phase of an emergency.

Pyrethroid impregnated top-sheets and traditional chaddars (cotton wraps used to sleep in) have been shown to exert deterrent and killing effects on malaria vectors in Pakistan (Rowland *et al.*, 1999). Their efficacy has been demonstrated against transmission of leishmaniasis (Reyburn *et al.*, 2001) and malaria (Rowland *et al.*, 1999). Permethrin-treated top-sheets are now, therefore, used operationally for control of malaria outbreaks in refugee camps and villages in Pakistan and Afghanistan. There appears to be great potential for the use of pyrethroid-impregnated blankets as a rapid, effective and easy-to-apply vector control measure for epidemics, disasters and conflict situations almost anywhere in the world.

Until now, among the range of pyrethroids used for ITNs (Zaim *et al.*, 2000), only permethrin has been evaluated for top-sheets (Rowland *et al.*, 1999). As patent protection of pyrethroids expires, malaria control authorities become increasingly reliant on generic insecticides that may fluctuate in quality, price and availability. Recent mergers between agrochemical companies have brought changes of product focus, whereby permethrin is no longer so readily obtainable as alpha-cyano pyrethroids (e.g. alphacypermethrin, deltamethrin, lambdacyhalothrin). Also, instead of the emulsion concentrate (EC) formulation, based on flammable hydrocarbons, less hazardous aqueous formulations of more potent pyrethroids have gained approval by the World Health Organization Pesticides Evaluation Scheme (WHOPES) www.who.int.ctd.html/whopes.html.

As SC formulations of deltamethrin and alphacypermethrin are already used as acceptable treatments for ITNs (Zaim *et al.*, 2000), evidence is needed of the efficacy of these insecticides compared to the tried-and-tested permethrin on top-

sheets. At appropriate dosages, deltamethrin SC has insecticidal efficacy similar (Curtis *et al.*, 1996) or superior (Miller *et al.*, 1999) to that of permethrin against anopheline mosquitoes. Alphacypermethrin SC at 40mg ai/m² was more effective than permethrin EC 500 mg ai/m² against *Anopheles gambiae* in hut trials and bioassays (Jawara *et al.*, 1998). At lower dosages lambdacyhalothrin has efficacy comparable to permethrin (Miller *et al.*, 1995, 1999; Jawara *et al.*, 1998;) and longer residual life than either permethrin or deltamethrin (Curtis *et al.*, 1996). However, the transient but uncomfortable nasal irritation and parathesia associated with use of lambdacyhalothrin (Njunwa *et al.*, 1991; Maxwell *et al.*, 1999) indicates probable unsuitability as a blanket treatment.

Although the safety of the pyrethroids discussed above are well documented (Zaim *et al.*, 2000; Barlow *et al.*, 2001), the possibility of adverse side-effects must be examined when considering personal protection methods that involve close contact. Equally important is the question of user preferences. Although the tool may be proved effective entomologically, it will only be efficacious if used on a regular basis by individuals at risk from malaria. Experience of side-effects will of course influence this.

A study comparing deltamethrin, alphacypermethrin and permethrin in entomological trials and a small-scale acceptability trial had been carried out by the non-governmental organisation, HealthNet International, in Pakistan in 1999. However, the data remained unexamined. Here, the data have been collated and analysed both to gather evidence of alternatives to the permethrin EC tested previously as well as to give an indication of the most suitable insecticide for use on blankets to inform the planning of further blanket evaluations.

6.2 Materials and Methods

The following methods were used to carry out the study. The design and data collection was not part of this thesis. The raw data were collated and analysed as part of this thesis.

6.2.1 Study location

Both the entomological evaluation by platform bioassay and the acceptability study were carried out at the HealthNet International field station in Azakhel refugee settlement. Insectary bioassays took place at the field station in Adizai refugee settlement. Both field stations are described in more detail above (Chapter 2, Section 2.1).

6.2.2 *Materials*

Sheets were impregnated using the following formulations and concentrations: permethrin ('Imperator' 25% EC: Zeneca, Fernhurst, U.K) at 500mg ai/m², deltamethrin ('K-Othrin' 5% SC: Aventis, Frankfurt, Germany) at 25mg ai/m², alphacypermethrin ('Fendona' 10% SC: American Cyanamid, Princeton, NJ, U.S.A) at 25mg ai/m², and a placebo treatment of 0.5% salt in water. Sheets were dried in the shade.

6.2.3 *Overnight platform bioassays*

Overnight platform bioassays were carried out following the method described above (Chapter 2, Section 2.2). In the centre of each platform, within the giant trap-net, two men slept on mattresses on wooden frame beds, their covering for the night being the sheets treated with one of the three pyrethroids or the placebo treatment. The men wore their normal clothing (shalwar chemise) beneath the sheets. The experiment was conducted over 16 nights. Each of the 4 treatments was evaluated on each of the four platforms on 4 separate occasions using 4 pairs of men in rotation.

6.2.4 *Insectary bioassays*

In order to examine the effectiveness of each insecticide under more controlled conditions and to examine the effect of washing, insectary bioassays were carried out. Four cotton shirt sleeves, each with a cotton glove sewn to the cuff, were impregnated with either of the three insecticide treatments or the placebo. To conduct the bioassay the sleeve was worn as normal and the tester placed his arm inside a holding cage housing 50 laboratory-reared, unfed 2-5 days old female *An. stephensi* for 15 minutes. All mosquitoes were then transferred to a humidified paper cup containing sugar solution. Numbers of mosquitoes blood-fed or unfed and dead or alive were recorded immediately and again after 12 hours.

Four people were used to test the sleeves with each person testing each treatment once on different days. After the first four-day rotation the sleeves were all washed with common bar soap (Lux™) at 4g/l for 2min, rinsed for 2min, and the rotation repeated.

6.2.5 *Acceptance study*

The popularity of the 4 treatments (3 pyrethroids and placebo) was tested by 88 families divided into 4 groups. All materials used as top-sheets and blankets at night

in each household were impregnated with the treatment to which their family had been randomly assigned. The families were requested to use these as normal for two weeks and not to wash them. At the end of the two weeks a responsible member of each family who would be aware of all family members' experiences (usually a mother or elder girl) was questioned using a structured questionnaire. Informed consent was obtained from all families recruited to the study.

6.2.6 Statistical Analysis

Entomological data were entered in Excel 98 and analysed in STATA 7. The proportion dead and the proportion blood-fed from the entomological studies were arcsine transformed to normalise the variance and subjected to analysis of variance and unpaired t-tests.

6.3 Results

6.3.1 Outdoor platform bioassays

Culicines were ~10 times more numerous than anophelines during the study period: 30,779 culicines and 3,014 anophelines were caught and released. The anophelines were mostly *An. nigerrimus* (45%), *An. stephensi* (27%), and *An. pulcherrimus* (14%). The remaining 13% were comprised of *An. subpictus*, *An. fluviatilis*, *An. annularis*, *An. culicifacies*, *An. splendidus* and *An. maculatus*. The majority of culicine captured were *Culex quinquefasciatus* and *Cx. tritaeniorhynchus*, whereas the remainder were mainly *Cx. bitaeniorhynchus* and *Cx. vishnui* were present in smaller numbers. An average of 528 ± 27 (mean \pm standard error, SE) mosquitoes were released per platform per night, comprising 481 ± 25 culicines and 47 ± 3.5 anophelines.

Table 6-1 shows the mean percentage mortality for each treatment type. Mortality at the sites with untreated top-sheets was lower for the culicines than for anophelines. Mean mortality fluctuated from 16% to 34% depending on the species considered. Figure 1 depicts the proportions blood-fed live, unfed live, blood-fed dead and unfed dead, after allowing 24 hours for delayed, insecticide-induced mortality. It is evident from Figure 6-1 that mortality rates were higher on the platforms with pyrethroid-impregnated sheets than on those with untreated sheets. This trend is significant for the anophelines as a genus and for *An. nigerrimus* as an individual species but low mosquito numbers affected the significance for some species. Culicine mortality was generally lower than anopheline mortality and the effect of pyrethroids, while

significant, was not as pronounced as against some anopheline species. There were no consistent or significant differences between the three pyrethroids on mortality.

Table 6-2 shows the mean percentage of blood-fed mosquitoes. Among culicines, the proportion blood-fed was significantly higher at the control site than at sites with treated sheets. Although anopheline blood-feeding appeared to be marginally lower at the treatment sites, this was only significant for the deltamethrin-treated sheet. Differences of impact between pyrethroids was significant only for *An. nigerrimus*, against which permethrin was less effective than the other two pyrethroids in preventing blood-feeding.

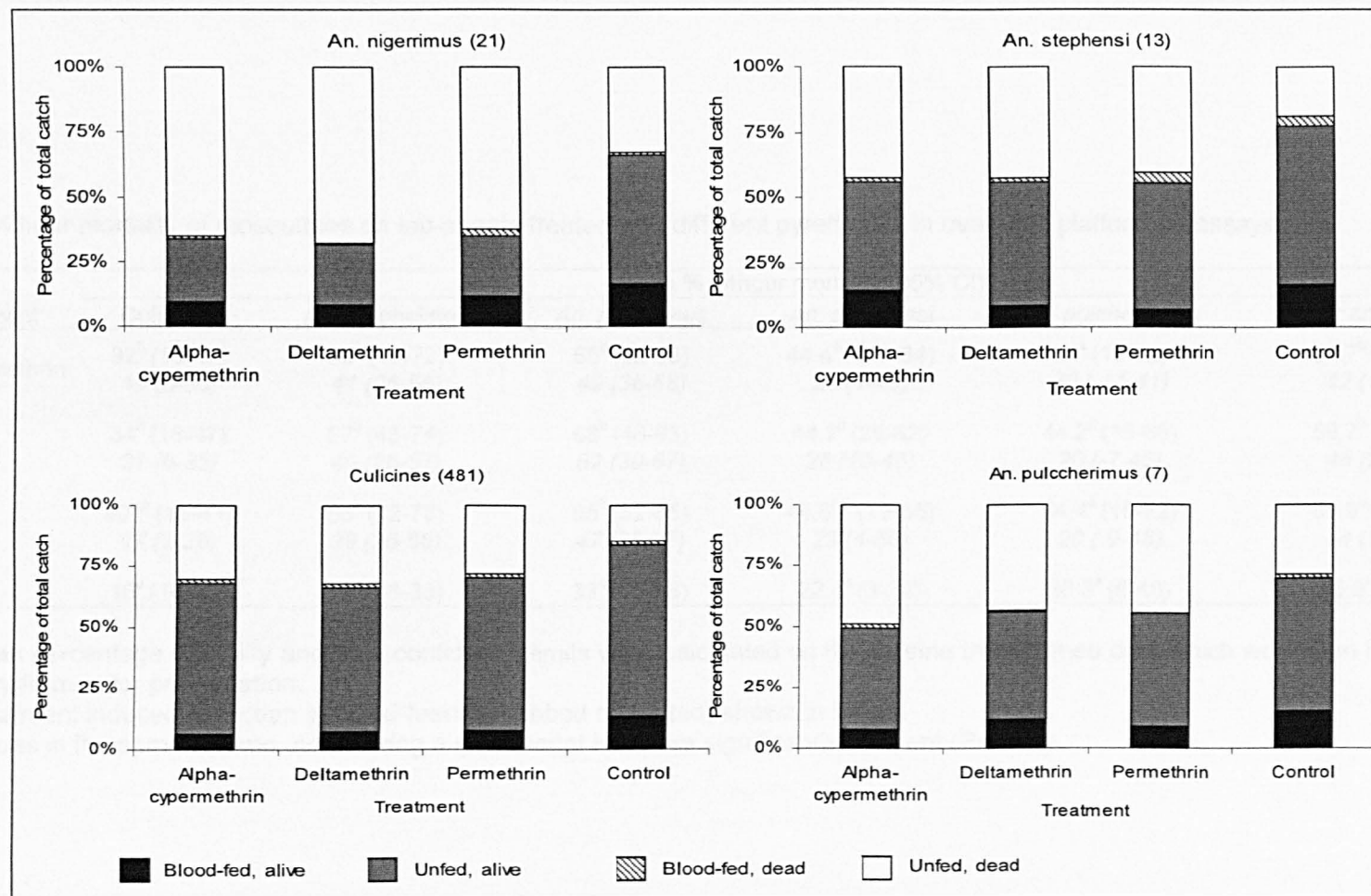


Figure 6-1. Condition of mosquitoes collected from outdoor platforms.
 Note: Mean number caught per platform per night shown in parentheses

Table 6-1. 24 hour mortality of mosquitoes on top-sheets treated with different pyrethroids in overnight platform bioassays

Net treatment	Mean % 24hour mortality (95% CI)					
	Culicines	All anophelines	<i>An. nigerrimus</i>	<i>An. stephensi</i>	<i>An. pulcherrimus</i>	Other anophelines
Alphacypermethrin	32 ^b (18-43)	58 ^b (43-72)	66 ^b (52-85)	44.4 ^{a,b} (17-64)	51 ^a (10-62)	57.7 ^b (27-79)
	19 (5-30)	41 (26-55)	49 (36-68)	28 (1-49)	30 (-11-41)	42 (12-64)
Deltamethrin	34 ^b (18-47)	57 ^b (43-74)	68 ^b (46-83)	44.2 ^b (25-62)	44.2 ^a (16-69)	59.7 ^b (35-81)
	21 (6-35)	40 (26-57)	52 (30-67)	28 (10-46)	20 (-7-45)	45 (21-67)
Permethrin	30 ^{a,b} (15-41)	56 ^b (42-73)	65 ^b (52-85)	44.6 ^{a,b} (19-66)	44.4 ^a (15-72)	58.9 ^b (27-84)
	17 (2-28)	39 (25-56)	47 (35-67)	29 (4-50)	20 (-9-48)	44 (13-69)
None	16 ^a (10-20)	29 ^a (16-38)	33 ^a (15-46)	22.4 ^a (3-35)	30.3 ^a (6-48)	26.6 ^a (4-37)

Notes:

1. Mean percentage mortality and 95% confidence limits were calculated on the arcsine transformed data which were then back transformed for presentation.
2. Treatment induced reduction in blood-feeding (Abbott corrected) shown in italics.
3. Values in the same column, not sharing a superscript letter are significantly different (P<0.05).

Table 6-2. Blood-feeding of mosquitoes on top-sheets treated with different pyrethroids in overnight platform trials

Net treatment	Mean % blood-fed (95% CI)					
	Culicines	All anophelines	<i>An. nigerrimus</i>	<i>An. stephensi</i>	<i>An. pulcherrimus</i>	Other anophelines
Alphacypermethrin	8.0 ^b (5.8-9.6) <i>45.6 (43-47)</i>	13.2 ^{a,b} (9.5-16.1) <i>25.8 (22-29)</i>	10.4 ^b (2.3-13.6) <i>39.9 (32-43)</i>	16.3 ^a (4.9-22.5) <i>17.3 (6-23)</i>	9.7 ^a (0.2-9.0) <i>41.2 (32-41)</i>	17.3 ^a (1.2-26.8) <i>-11.6(-28-2)</i>
Deltamethrin	9.2 ^b (6.7-11.0) <i>37.4 (35-39)</i>	10.4 ^b (5.4-13.5) <i>41.6 (37-45)</i>	8.7 ^b (1.7-10.5) <i>49.7 (43-52)</i>	10.1 ^a (2.9-13.3) <i>48.7 (42-52)</i>	12.3 ^a (0.6-15.1) <i>25.5 (14-28)</i>	10.6 ^a (0.9-12.2) <i>31.6 (22-33)</i>
Permethrin	9.5 ^b (6.4-11.8) <i>35.4 (32-38)</i>	12.3 ^{a,b} (9.0-14.8) <i>30.9 (28-33)</i>	14.6 ^a (11.9-16.8) <i>15.6 (13-18)</i>	11 ^a (2.6-14.4) <i>44.2 (36-48)</i>	9 ^a (0.2-10.5) <i>45.5 (37-47)</i>	6.3 ^a (0.0-6.4) <i>59.4 (53-59)</i>
None	14.7 ^a (10.2-18.2)	17.8 ^a (11.4-22.3)	17.3 ^{a,b} (4.6-23.2)	19.7 ^a (5.9-26.4)	16.5 ^a (4.0-22.0)	15.5 ^a (2.6-17.7)

Notes:

1. Mean percentage blood-fed and 95% confidence limits were calculated on the arcsine transformed data which were then back transformed for presentation.
2. Treatment induced reduction in blood-feeding (Abbott corrected) shown in italics.
3. Values in the same column, not sharing a superscript letter are significantly different (P<0.05).

6.3.2 Insectary bioassays

The insectary bioassays concurred with the results of the outdoor platform bioassays in showing no significant difference between the three treatment types on mortality and blood-feeding. Each treatment type showed a significant increase in mortality and reduction of blood-feeding compared to the untreated sleeve (Figure 6-2). After one wash no significant differences were seen between this untreated control and the treatments.

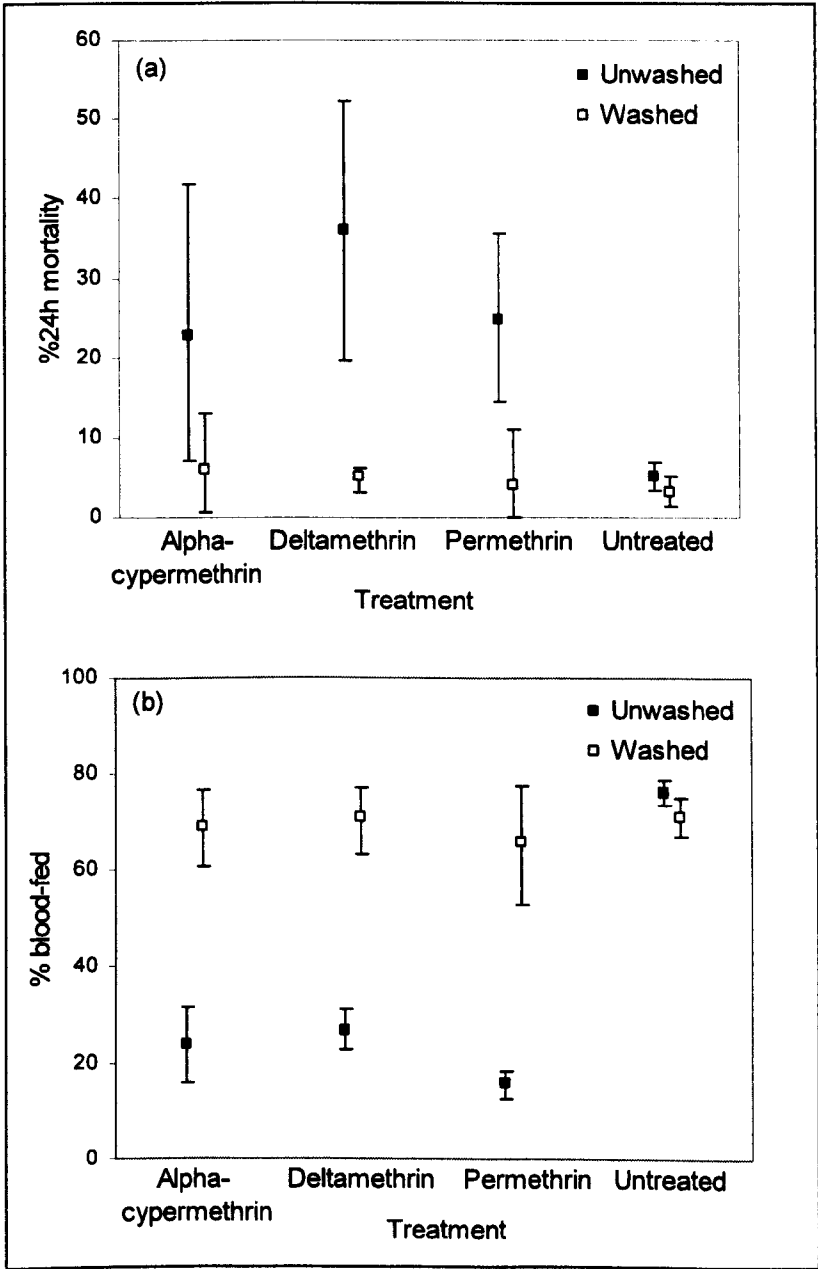


Figure 6-2. Insectary bioassays on washed and unwashed pyrethroid-impregnated sleeves. (a) mean percentage mortality 24 hours after exposure; (b) mean percentage blood-fed.

Note: Means and 95% CIs were calculated on arcsine transformed data then back-transformed for presentation.

6.3.3 Acceptance study

Table 6-3 shows the results of the questionnaire in which users of treated sheets were asked about their experiences and opinions. Only minor side-effects were reported; these included general and localized itching, redness or rash, and sneezing. The number of families reporting any-side effects was small (6/88). No complaints of side-effects arose from families sleeping under sheets impregnated with alphacypermethrin or permethrin, and all of these families said they appreciated the treated sheets. However, in the group of families using deltamethrin 5/21 families reported various minor side-effects and six families said they disliked the treated sheets. In the placebo group one family reported side-effects (general skin itching) and only 52% stated that they liked the intervention.

Table 6-3. Responses of users to the acceptability questionnaire comparing top-sheets treated with different pyrethroid insecticides or untreated

	Deltamethrin (n=21)	Permethrin (n=24)	Alphacypermethrin (n=22)	Placebo (n=21)
Any side-effects	5	0	0	1
General itching	3	0	0	1
Skin itching	3	0	0	1
Eye itching	3	0	0	0
Nose itching	2	0	0	0
Sneezing	2	0	0	0
Redness or rash	1	0	0	0
Did you like it?	15	24	22	11
Did you wash it?	1	2	0	0

Note:

Figures shown are the number of respondents replying affirmatively when asked if they had experienced any side-effects, liked the sheets or had washed the sheets are shown, as well as any particular side-effects mentioned when respondents were asked to specify.

6.4 Discussion

No consistent significant difference was seen in this evaluation between the insecticidal efficacy of the three pyrethroids tested, either in outdoor platform bioassays with wild mosquitoes, or in fixed-time exposure bioassays with insectary reared mosquitoes. This is an encouraging result as it indicates that this protection method is adaptable in terms of the type of insecticide used, with both alphacypermethrin and deltamethrin achieving similar results to those previously demonstrated for permethrin (Rowland *et al.*, 1999). It had been shown previously that sleeping under sheets impregnated with permethrin can provide 64% (CI=35%-80%) protective efficacy against *Plasmodium falciparum* and 38% (CI=0%-64%) protection against *P. vivax* amongst children and teenagers (Rowland *et al.*, 1999). The comparable performance of alphacypermethrin and deltamethrin to permethrin suggests that these may also have potential as treatments for top-sheets or blankets. The sudden loss of insecticidal efficacy after just one wash was disappointing. Cotton generally retains pyrethroids less effectively than polyethylene or polyester (Luo Dapeng *et al.*, 1994). If this control tool is to be effective operationally it is extremely important that a pyrethroid is used which does not produce irritant effects, as this may prompt users to wash the sheets or blankets.

Adverse side-effects have more often been reported from deltamethrin use than from alphacypermethrin or permethrin use (Sexton, 1994), and this was borne out in our study. No complaints of side-effects arose from families using the permethrin or the alphacypermethrin sheets, yet a significant number did complain of irritation when using the deltamethrin treatment. Of the three respondents who reported having washed their sheets (despite being asked not to), two of these had been allocated to the deltamethrin group and had reported side-effects. The possibility that it was the irritation caused by the insecticide that prompted the users to wash the sheets is a serious concern. It is worrying to note that the nine users of deltamethrin who stated that they disliked the treated blankets gave the reason for this as being 'no noticeable prevention in mosquito biting'. This statement contradicts the entomological findings. Two of these users were the deltamethrin respondents who admitted to having washed their sheets. It is a possibility that others of the deltamethrin group were also prompted by the uncomfortable side-effects to wash their sheets but failed to admit to it. This may have been the cause of the surprising number of deltamethrin users perceiving the sheets as ineffective at repelling or killing mosquitoes.

In contrast to the deltamethrin and placebo users, all families using the permethrin and alphacypermethrin treatments said they liked the impregnated sheets, citing reduction in mosquito biting as the reason. It is generally considered that methods of personal protection

must have a significant effect against the more numerous, nuisance culicines in order to be appreciated by users (Guillet *et al.*, 2001). Here, the impregnated sheets did have a significant effect on culicine mortality and feeding rates, although, as is generally the case with pyrethroids (Curtis *et al.*, 1996; Maxwell *et al.*, 1999) it was a less substantial effect than against anophelines. It is encouraging that, despite this, users were able to notice a beneficial effect suggesting that when implemented operationally it would be well used.

In addition to reducing malaria transmission and providing some protection from nuisance biting, a control tool such as this should also be successful in reducing transmission of cutaneous leishmaniasis (CL) by phlebotomine sandflies (Diptera: Psychididae). Permethrin-treated materials are impressive for reducing sandfly densities indoors: *Sergentomyia* spp. (Majori *et al.*, 1989), *Phlebotomus papatasi* Scopli (El naiem *et al.*, 1999) and *Phlebotomus perfiliewi* Parrot (Maroli & Lane, 1987). Permethrin-treated top-sheets can be just as effective as ITNs for protection against leishmaniasis (both achieving about 65% protective efficacy), as demonstrated in a household randomised trial in Kabul (Reyburn *et al.*, 2000). Further evaluations of this control method against CL, perhaps using alphacypermethrin, the most promising alternate insecticide evaluated here, are warranted.

For this entomological study impregnated sheets were evaluated, as these are the materials more commonly used as a night covering in this region in the summer months when most malaria transmission occurs. This tool has exciting potential if adapted for more widespread complex emergency situations. Blankets and quilts are among the first commodities to be distributed to refugees or IDPs, alongside food and shelter. If blankets and quilts (made of wool/cotton/synthetic mix) were impregnated with a pyrethroid insecticide before distribution, possibly incorporating a long-lasting wash-resistant treatment (N'Guessan *et al.* 2001), large-scale coverage of personal protection could be achieved rapidly.

Blankets with insecticide treatment are likely to be as effective in killing or deterring malaria vectors as the treated top-sheets. It is clear that washing reduces the insecticidal activity of treated sheets. Treated blankets may not be washed as frequently or vigorously as treated top-sheets because blankets are bulkier, darker in colour and slower to appear dirty. Alternatively, long lasting insecticidal netting impregnated with an appropriate repellent pyrethroid might be sewn onto the outer side of blankets during production. Treating blankets with insecticide by dipping is a possible tool suitable for use now in the early acute stages of an emergency. In the meantime methods which may extend the insecticidal life of an insecticide-treated blanket should be examined further.

Chapter 7. An entomological evaluation of prototype insecticide-treated blankets: tested when used alone or in combination with insecticide-treated plastic sheeting

7.1 Introduction

The use of pyrethroid treated top-sheets (cotton sheets used for cover when sleeping) has been shown to exert deterrent and killing effects on malaria vectors in Pakistan (Rowland *et al.*, 1999). Their efficacy has been demonstrated against transmission of cutaneous leishmaniasis (Reyburn *et al.*, 2000) and malaria (Rowland *et al.*, 1999; Macintyre *et al.*, 2003). Permethrin treatment of top-sheets is now used operationally for controlling outbreaks of malaria in refugee camps and villages in Pakistan and Afghanistan. A study examining the potential of alternative pyrethroids to permethrin as top-sheet treatments showed that deltamethrin, alphacypermethrin and permethrin all showed equally good mortality and deterrence, but users of the deltamethrin sheets reported some skin irritation (5 of 21 families) which was not reported in any of the permethrin or alphacypermethrin users (Graham *et al.*, 2002b; Chapter 6). The same study showed that just one hand wash could greatly reduce the efficacy of such material in insectary bioassays, a severe limitation for achieving medium-term control in operational use which currently limits the application of this tool to use in outbreak response.

In complex emergencies blankets are distributed to the affected population as one of the priority items. If these blankets were treated with insecticide and shown to exert deterrent and/or killing effects in the same way as top-sheets treated with insecticide have been shown to do, then these may be a useful tool for malaria control. Unlike insecticide-treated nets or shelter material, blankets can be used regardless of whether the people sleep indoors or outdoors, with no need for support.

In order to extend their insecticidal life the first prototype blankets developed (and tested here) incorporated the long-lasting treatment technology, PermaNet™, which had already been developed by the manufacturer of Vestergaard Frandsen (WHOPES, 2004a), a polyester mesh surface-treated with deltamethrin in a resin. To keep the cost of the treated blanket down and to guard against sustained skin contact with the pyrethroid it was decided that the mesh would be attached to one side of the blanket only. The blanket therefore must be used with the treated side facing outer-most in

order to provide protection. Two concerns were raised following the development of this deltamethrin prototype blanket i) that a less irritant pyrethroid than deltamethrin should be used (Graham *et al.*, 2002a; Chapter 6), and ii) that the blankets may be used “incorrectly” with the treated side turned to face the body, as the soft polyester mesh attached to one side of the blanket resembled quilting. A second prototype blanket was therefore suggested to Vestergaard and developed by them whereby permethrin-impregnated polyethylene yarn, which has a rougher texture than polyester, was needle-punched over one side of the blanket. The technology of incorporating permethrin into polyethylene before extrusion is used to make the WHOPES approved Olyset® long lasting net (Itoh & Okuno, 1996; N’Guessan *et al.*, 2001; WHO, 2001a; Tami *et al.*, 2004). However, the blankets tested here were manufactured by a different company, the permethrin-impregnated polyethylene yarn used in these blankets has not, therefore, been independently evaluated for efficacy and resistance to washing.

Presented here are three trials examining, in both 3 minute exposure bioassay and overnight platform tests: i) the first prototype blanket with deltamethrin/polyester mesh; ii) a similar blanket hand-made from cut-up PermaNet and created to attempt to replicate the results of the previous year’s tests, and iii) the second prototype blanket with permethrin/polyethylene yarn.

Additional overnight platform tests were performed to examine the use of treated blankets by sleepers also protected by insecticide-treated plastic sheeting. Existing evidence (Graham *et al.*, 2002b; Chapter 3; Chapter 4) suggests that although insecticide-treated plastic sheeting may not provide personal protection from biting, it may be useful as a malaria control tool through a mass killing effect on the vector population, when good coverage of a settlement is achieved. Top-sheets treated with insecticide have been shown to provide good personal protection from biting mosquitoes. With both blankets and shelter material distributed at the outset of emergencies in a camp environment a combination of these tools treated with insecticide could potential provide excellent protection from malaria by resulting in a mass-effect on the mosquito population and by providing personal protection from biting for those individuals sleeping under the treated blankets.

7.2 Materials and Methods

7.2.1 Study location

Insectary bioassays were carried out at the HealthNet International field site in Adizai refugee settlement. Overnight platform trials took place at the HealthNet International entomological field station in Azakhel refugee settlement. Both field stations are described in more detail above (Chapter 2, Section 2.1)

7.2.2 Materials

Items tested were:

- i) Woollen blankets with PermaNet mesh attached to one side during manufacture. PermaNet is a mesh made of polyester multifilament (denier 100, mesh 156 holes/inch²), surface-treated with deltamethrin at 45-55 mg a.i./m² in a resin formation (WHO 2004a).
- ii) Woollen blankets with PermaNet mesh hand-sewn to one side prior to testing (hand-constructed on site).
- iii) Woollen blankets with permethrin-impregnated polyethylene yarn sewn over one side. The permethrin was incorporated into the polyethylene during manufacture to a target dose of 28g a.i./kg polyethylene. This polyethylene was then extruded into yarn which was attached to one side of the blanket by needle punching the yarn into the wool in an irregular pattern.
- iv) Untreated woollen blankets of the same specifications as those tested with insecticide treatments, provided by the manufacturer.
- v) Black and white insecticide-treated plastic sheeting: a fore-runner to the commercially available Zerofly™, described further in Chapter 3.
- vi) Zerofly™ insecticide-treated plastic sheeting, described further in Chapter 3.

All items were manufactured by Vestergaard Frandsen, Denmark.

7.2.3 Insectary Bioassays

Fixed-time exposure bioassays (3 minutes) and median time to knockdown bioassays were carried out using the standard methods described above in Chapter 2, section 2.2.

7.2.4 Overnight Platform Bioassays

The following combinations of items were tested:

Trial 1 (2002, November – tail-end of mosquito season)

- 1. Blanket with PermaNet mesh (factory made)**
- 2. Black and white prototype plastic sheeting + Blanket with PermaNet mesh (factory made)**
- 3. Untreated blanket**
- 4. Untreated blanket + Untreated plastic sheeting**

Trial 2 (2003, September – peak mosquito season)

- 1. Blanket with PermaNet mesh (hand-sewn)**
- 2. Zerofly™ sheeting + Blanket with PermaNet mesh (hand-sewn)**
- 3. Zerofly™ sheeting + Untreated blanket**
- 4. Untreated blanket**

Trial 3 (2003, November – tail-end of mosquito season)

- 1. Permethrin / polyethylene blanket**
- 2. Untreated blanket**

Overnight platform bioassays were carried out following the method described above (Chapter 2, Section 2.2). Two or four men slept on each platform on a bedroll on the floor wearing normal clothing (shalwar chemise) and covered with an untreated or treated blanket (in trials 1 and 2 two men slept on each platform with one blanket each; in trial 3 four men slept on each platform with each pair sharing one blanket).

Where plastic sheeting was used these were constructed as Λ -shaped shelters using a ridge-pole and 2 upright poles. The shelters were open at each end and pegged to the floor along the edges.

7.2.5 Statistical analysis

Data were entered in Excel 98 and analysed using STATA 6 software. Proportional data (KD, mortality and blood-feeding) from the platform trials and the 3-minute fixed-time exposure bioassays were analysed using blocked logistic regression. Comparisons between treatments were made by successively dropping treatments from the overall comparison. This process allowed each treatment to be compared with every other. Means and confidence limits of the constant for each treatment were back transformed for presentation as follows.

$$x' = \frac{1}{1 + (1/\exp(x))}$$

Where: x' = back-transformed value
 x = the value from the logistic regression

7.3 Results

7.3.1 Insectary bioassays

In fixed-time contact bioassay a similar proportion of mosquitoes were knocked-down 1h after exposure to both the deltamethrin/polyester and the permethrin/polyethylene treatment. Low 24-hour mortality on the permethrin/polyethylene treatment indicates considerable recovery of knocked-down mosquitoes during the 24h holding period; no such recovery was seen in mosquitoes exposed to the deltamethrin/polyester blanket, in which there was almost 100% mortality after 24 hours. Median time to knockdown was almost 50% longer for the permethrin/polyethylene treatment than for the PermaNet treatment.

Table 7-1. 1 hour Knockdown and 24 hour mortality following 3 minute exposure and median time to knock-down of mosquitoes exposed to blanket treatments

	Number of replicate tests	% 1hr KD (95% CI)	% 24hr mortality (95% CI)	MTKD seconds (95% CI)
Deltamethrin / polyester blanket	20	93.0% (88.5 - 95.8)	99.5 % (96.5 – 99.9)	439.0 (403.7 - 474.3)
Permethrin / polyethylene blanket	12	94.2% (88.3 - 97.2)	62.5% (53.5 – 70.7)	649.0 (524.6 - 773.4)

Note: In fixed-time exposure bioassays batches of 10 or 11 mosquitoes were used

7.3.2 Overnight platform tests

A total of 281.9 (± 9.1) culicines and 52.6 (± 4.9) anophelines (\pm standard error) were caught per platform per night. The majority of the anopheline mosquitoes were *An. subpictus* (29.2 ± 3.9), *An. culicifacies* (12.7 ± 1.2) and *An. stephensi* (6.6 ± 0.6), with smaller numbers of *An. annularis*, *An. pulcherrimus*, *An. splendidus* and *An. fluviatilis* also present.

Mosquito mortality on platforms with untreated items was fairly high in all trials, especially in trial 3.

Culicine blood-feeding was similar in all trials. In trial 1 there was a slightly higher proportion of anopheline blood-feeding than in trials 2 and 3. In all trials the proportion of culicines blood-fed was lower than the proportion of blood-fed anophelines.

In trial 1 a significant increase in mosquito mortality was seen when men slept under deltamethrin/polyester treated blankets rather than untreated blankets (Table 7-2). This held true for both genera; treatment induced mortality was 45.5% for culicines and 40.1% for anophelines (both $P < 0.001$). However, in the repeated trial the following year (which took place earlier in the mosquito season) both culicine and anopheline mortality was similar on both treated blankets and untreated blankets.

Although in trial 3 there is a trend towards higher anopheline mortality on the permethrin/polyethylene treated blanket (Table 7-2) the difference between the means is fairly small and the confidence intervals around these means are wide. There is no difference in culicine mortality when men sleep under untreated blankets compared to when they sleep under permethrin/polyethylene treated blankets.

Mosquito mortality is significantly higher when men sleep under plastic sheeting and use a treated blanket than when men sleep under treated blankets alone. This is true for both culicines and anophelines (Table 7-2). In trial 2 however the use of a treated blanket *alone* did not demonstrate increased mosquito mortality. The elevated mortality seen when the treated blanket is used by men sleeping under Zerofly™ plastic sheeting appears to be purely the effect of the Zerofly™ sheeting, similar mortality is seen when the Zerofly™ is used with an untreated blanket.

Use of either a deltamethrin or permethrin treated blanket *alone* does not provide the sleepers with protection from biting in any of the trials, either from anopheline or culicine mosquitoes (Table 7-3). In trial 2 there is some indication that men sleeping

under Zerofly™ plastic sheeting with a treated blanket are bitten less than those sleeping under Zerofly alone, and for culicine mosquitoes the lowest biting rate seen in all three trials is on men sleeping under the Zerofly sheeting whilst covered with deltamethrin treated blankets.

Table 7-2. 24 mortality of mosquitoes on treated and untreated blankets with and without treated sheeting, in overnight platform tests with human sleepers.

		No. of nights	Culicines			Anophelines ⁴		
			No. of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality	No. of mosq. per night Mean (SD)	% mortality (95% CI) ²	Treatment induced % mortality
TRIAL 1 – Nov 2002	Treated blanket (PermaNet mesh)	15	294.5 (70.5)	24.7% ^a (23.5 - 26.0)	10.5%	61.9 (48.0)	49.2% ^a (46.0 - 52.4)	30.9%
	Untreated blanket	15	267.9 (67.1)	15.9% ^b (14.8 - 17.1)	-	42.5 (23.3)	26.5% ^b (23.2 - 30.1)	-
	Treated blanket (PermaNet mesh) + Treated sheeting (black and white)	15	275.7 (67.6)	52.0% ^c (50.5 - 53.5)	45.5%	52.5 (39.9)	58.0% ^c (54.5 - 61.4)	41.9%
	Untreated blanket + Untreated sheeting	21	305.0 (73.5)	11.9% ^d (11.1 - 12.7)	-	51.1 (34.3)	27.7% ^b (25.1 - 30.5)	-
TRIAL 2 – Sept 2003	Treated blanket (PermaNet mesh)	25	227.2 (42.7)	8.0% ^a (7.3 - 8.7)	0.9%	42.5 (27.0)	12.1% ^a (10.3 - 14.2)	No induced mortality
	Untreated blanket	24	227.5 (47.5)	7.2% ^a (6.5 - 7.9)	-	37.6 (21.5)	14.7% ^a (12.6 - 17.2)	-
	Treated blanket (PermaNet mesh) + Zerofly™	25	227.5 (60.7)	13.7% ^b (12.8 - 14.6)	No induced mortality	32.8 (17.5)	23.5% ^b (20.8 - 26.6)	2.4%
	Untreated blanket + Zerofly™	25	237.9 (63.3)	13.9% ^b (13.0 - 14.8)	-	34.0 (24.7)	21.6% ^b (19.0 - 24.5)	-
TRIAL 3 – Nov 2003	Treated blanket (permethrin /polyethylene)	12	80.6 (22.2)	28.3% ^a (26.0 - 30.7)	No induced mortality	5.2 (3.7)	25.8% ^a (17.9 - 35.6)	8.5%
	Untreated blanket	6	91.3 (15.6)	28.9% ^a (26.3 - 31.7)	-	3.1 (2.2)	18.9% ^a (9.3 - 34.7)	-

Notes:

1. Percentage mortality and 95% confidence limits were back-transformed from values calculated by the blocked logistic regression model.
2. Within each trial and genera, rows not sharing a superscript letter differ significantly ($P < 0.05$) in the blocked logistic regression model.
3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used, wild-caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included merely to indicate the size of the denominator for calculation of percentages. These means do not differ significantly by Scheffe's multiple comparison test.
4. Due to low numbers of individual species all anophelines have been grouped.

Table 7-3. Blood-feeding of mosquitoes on treated and untreated blankets with and without treated sheeting, in overnight platform tests with human sleepers.

		No. of nights	Culicines			Anophelines ⁴		
			No. of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood-feeding	No. of mosq. per night Mean (SD)	% blood-fed (95% CI) ²	Treatment induced % reduction in blood-feeding
TRIAL 1 – Nov 2002	Treated blanket (PermaNet mesh)	15	294.5 (70.5)	4.4% ^a (3.9 - 5.1)	No reduction	61.9 (48.0)	34.1% ^a (31.1 - 37.2)	No reduction
	Untreated blanket	15	267.9 (67.1)	3.9% ^a (3.4 - 4.6)	-	42.5 (23.3)	25.9% ^b (22.6 - 29.4)	-
	Treated blanket (PermaNet mesh) + Treated plastic (black and white)	15	275.7 (67.6)	4.6% ^a (4.0 - 5.3)	No reduction	52.5 (39.9)	37.8% ^a (34.5 - 41.3)	No reduction
	Untreated blanket + Untreated sheeting	21	305.0 (73.5)	4.1% ^a (3.7 - 4.7)	-	51.1 (34.3)	21.3% ^c (19.0 - 23.9)	-
TRIAL 2 – Sept 2003	Treated blanket (PermaNet mesh)	25	227.2 (42.7)	3.8% ^a (3.4 - 4.4)	No reduction	42.5 (27.0)	18.6% ^a (16.4 - 21.1)	2.1%
	Untreated blanket	24	227.5 (47.5)	3.9% ^a (3.4 - 4.5)	-	37.6 (21.5)	19.0% ^a (16.6 - 21.7)	-
	Treated blanket (PermaNet mesh) + Zerofly™	25	227.5 (60.7)	1.4% ^b (1.1 - 1.7)	65.0%	32.8 (17.5)	11.0% ^b (9.0 - 13.3)	3.5%
	Untreated blanket + Zerofly™	25	237.9 (63.3)	4.0% ^a (3.5 - 4.5)	-	34.0 (24.7)	11.4% ^b (9.4 - 13.7)	-
TRIAL 3 – Nov 2003	Treated blanket (permethrin /polyethylene)	12	80.6 (22.2)	8.1% ^a (6.8 - 9.7)	19.0%	5.2 (3.7)	18.3% ^a (11.7 - 27.5)	No reduction
	Untreated blanket	6	91.3 (15.6)	10.0% ^a (8.3 - 11.9)	-	3.1 (2.2)	13.5% ^a (5.7 - 28.6)	-

Notes:

1. Percentage mortality and 95% confidence limits were back-transformed from values calculated by the blocked logistic regression model.
2. Within each trial and genera, rows not sharing a superscript letter differ significantly ($P < 0.05$) in the blocked logistic regression model.
3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used, wild-caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included merely to indicate the size of the denominator for calculation of percentages. These means do not differ significantly by Scheffe's multiple comparison test.
4. Due to low numbers of individual species all anophelines have been grouped.

7.4 Discussion

These prototype blankets were developed with the aim of creating an efficacious vector control tool that could be delivered with no further effort other than the usual blanket distribution process and be protective against malaria over the first few weeks or months of an emergency. The focus was therefore on blankets that were pre-treated and were treated with insecticide in long-lasting formulations. The only two WHOPES recommended long-lasting insecticide formulations are the PermaNet™ deltamethrin/polyester mosquito net and the Olyset permethrin/polyethylene mosquito net. The prototype blankets therefore attempted to link this existing long-lasting technology to a blanket treatment by attaching a long-lasting polyester or polyethylene treatment to the surface of a normal untreated blanket. This was preferred to conventionally dipping or spraying the blankets with insecticide which, although expected to provide effective protection useful for epidemic response (Rowland *et al.*, 1999), is likely to rapidly loss efficacy once the material is washed (Graham *et al.*, 2002b, Chapter 6).

The results for this first prototype blanket, using PermaNet™ technology, were encouraging; although no reduction in blood feeding was demonstrated there was a 50% increase in culicine mortality and almost a doubling of anopheline mortality over that seen when men slept under untreated blankets. Permethrin treated top-sheets tested previously at the same site also showed a similar increase in mortality of the three predominant anophelines and of culicines, with mortality increased by between 75% - 275% over that on untreated sheets (Rowland *et al.*, 1999).

Unfortunately, the result achieved here in trial 1 could not be replicated the following year when blankets with PermaNets cut up and hand-sewn onto one side were constructed on site. One issue which may have effected the protection provided by these blankets is that the PermaNet mesh became slightly dislodged on a few of the blankets, meaning a fully insecticidal surface may not have been presented to the host-seeking mosquitoes.

Rowland and colleagues (1999) demonstrated a reduction in blood-feeding in culicine mosquitoes and two of the three most predominant anophelines, although the baseline anopheline blood-feeding was only half that seen in the current trial 1. It is therefore surprising that the current study failed to demonstrate a protective effect of the treated blankets. This PermaNet-type blanket treated requires further follow-up studies with

properly constructed blankets and perhaps tested over a longer period to allow collection of a more robust dataset to examine both mortality and blood-feeding.

It is clear from the insectary bioassays on the permethrin/polyethylene blankets that the manufacturing process failed to achieve sufficient bio-available concentrations of permethrin at the surface of the yarn: although the majority of mosquitoes were knocked-down on exposure, the dose of insecticide received was low enough to allow many of these mosquitoes to recover in the 24h holding period, and although permethrin is normally faster acting than deltamethrin (Hougard *et al.*, 2002) the permethrin-impregnated polyethylene yarn took twice as long to knock down mosquitoes than the deltamethrin-treated mesh. It is therefore unsurprising that little difference in mosquito mortality or blood-feeding is seen when men sleep under these blankets as compared to untreated blankets.

The benefits of using the rougher polyethylene are based on the presumption that blanket owners would be more likely to turn polyester surfaces towards their bodies and less likely to do so with polyethylene. It would be useful to carry out some simple user behaviour and preference surveys to document this. If polyethylene does seem the most appropriate substrate for the insecticide then further development of this tool is desirable. Vestergaard Frandsen's permethrin/polyethylene technique had not been previously independently evaluated for its efficacy in insectary bioassays or for the wash resistance of the treatment and it appears that they have not yet perfected this process. Sumitomo, the Japanese company which manufactures Olyset, a permethrin-impregnated polyethylene treatment with proven efficacy, have been approached to develop a blanket incorporating the Olyset technology.

The importance of ensuring a blanket with only one side treated will be used "correctly" stems from the stated objective of creating a vector control tool that can be delivered with no further effort than that already employed in the distribution of blankets. If the "correct" use of the blanket needs to be explained to the owners at the time of delivery this objective has not been met. Dipping or spraying the blankets, as is done with sheets for epidemic response has the benefit of treating the whole item with insecticide negating the issue of how the blanket will be orientated by the user, however, to-date only conventional insecticide treatments, which lose efficacy after washing were available. "Long-lasting" treatments in a tablet formulation are now commercially available (Kotab-123 Bayer, Germany) but have not yet completed WHOPES testing. If

these are approved by WHOPES as proven long-lasting treatments they may be an option for a dipping treatment of blankets. However, a disadvantage of a dipped blanket, which has insecticide treatment on both sides means there will be sustained contact with the skin. The current KOTab-123 treatment is a deltamethrin formulation. A similar permethrin formulation would be more suitable for a long-lasting blanket treatment.

It is proven that sleeping under treated materials provides protection from both malaria and from leishmaniasis (Rowland *et al.*, 1999; MacIntyre *et al.*, 2003) and further work to develop a pre-treated and long-lasting blanket should be carried out.

Chapter 8. Entomological evaluations of candidate long-lasting insecticide-treated nets

8.1 Introduction

Insecticide-treated nets (ITNs) are commonly used to reduce the risk of malaria transmission. Periodic re-treatment of ITNs with pyrethroid insecticide is necessary for their continued effectiveness against anopheline mosquitoes (Lines, 1996). Removing the need for re-treatment would circumvent a major operational problem faced by net owners and by ITN projects based on marketing re-treatment. In a complex emergency setting with a transient population the task of reaching net owners for retreatment is especially problematic, particularly when nets are provided to refugees or IDPs who then return to areas outside the reach of treatment services. Some manufacturers of chemicals and textiles are developing treatment processes in which the insecticide is more stably bound to fabrics (WHO, 2004b), resulting in long-lasting insecticidal nets (LLINs). The aim is to develop treatments that can resist washing and which enable nets to remain efficacious for their useful lifespan, often cited as 4-5 years. The first type of LLIN recognised by the World Health Organization Pesticide Evaluation Scheme (WHOPES) was the Olyset Net® (Sumitomo Chemical Co., Osaka, Japan), a wide-mesh polyethylene net with permethrin 2% w/w incorporated during manufacture (Itoh & Okuno, 1996; N'Guessan *et al.* 2001; WHO, 2001a; Tami *et al.*, 2004).

PermaNet™ is another type of LLIN, made of polyester surface-treated with deltamethrin 55 mg ai/m², developed by Vestergaard Frandsen A/S (Kolding, Denmark). Field trials of PermaNet manufactured in 2000-2001 have given inconsistent bioassay results (WHO, 2004a), showing efficacy persisting after washing in one South American study (Gonzales *et al.*, 2002), but showing efficacy declining with successive washes in one African study (Müller *et al.*, 2002). Therefore, during 2002 the manufacturer launched 'PermaNet™ 2.0'; the manufacturing process remained unchanged but measures were taken to overcome the earlier variability by improving quality assurance during manufacture (WHO, 2004a). This report includes evaluations of both versions of PermaNet compared to conventionally treated ITNs subjected to equivalent washing regimes. Without such controls any claim of improved wash-fastness lacks proof. Among published studies fulfilling this criterion, Gonzales *et al.* (2002) tested PermaNet in parallel with conventional polyester ITNs of three treatment types (alphacypermethrin

suspension concentrate (SC) 40 mg ai/m² or deltamethrin 25 mg ai/m² using SC and tablet formulations), and found no difference in insecticidal efficacy after three gentle washes but, with more aggressive washing, PermaNet outperformed the conventional ITNs. Conversely, Asidi *et al.* (2004) found no improved performance of PermaNet over ITNs conventionally treated with alphacypermethrin SC 20 mg ai/m², lambda-cyhalothrin CS (capsule suspension, microencapsulated) 18 mg ai/m² or permethrin EC (emulsifiable concentrate) 500 mg ai/m² after 5 washes and 8 months of use.

Dawa Net is a net manufactured by SiamDutch (Bangkok, Thailand) and, at the time of testing, was marketed as an LLIN. This net is also made of polyester which is surface-treated with deltamethrin. No studies have examined the wash fastness of this net.

To further elucidate the vital issue of PermaNet performance relative to that of conventionally treated nets under various field conditions this chapter includes data collected by the author in Pakistan and London (evaluations of both PermaNet and PermaNet 2.0) and compares these to data on the first version of PermaNet collected by two other research groups in Iran and Tanzania. The candidate long-lasting net, Dawa, is also evaluated. In all studies nets were compared to ITNs conventionally treated with insecticide, both local and standardised wash procedures were used and evaluation was by standard insectary and overnight bioassay methods.

8.2 Materials and Methods

The Pakistan based field work (insectary bioassay and overnight platform tests) as well as the insectary and laboratory testing carried out in London, on the first versions of PermaNet, PermaNet 2.0 and Dawa were all carried out by the author. The evaluations of the first version of PermaNet which took place in Iran and Tanzania were carried out without the involvement of the author. The details of these separate trials are included in this methodology section to provide the necessary background to the data which are analysed and presented in this chapter alongside the Pakistan and London work for comparison.

8.2.1 Study sites

Study sites have been described elsewhere for Muheza-Ubwari, Tanzania (Curtis *et al.*, 1996), for Kazeroun, Iran (Kayedi, 2004) and for Peshawar, Pakistan (Chapter

2, section 2.2). Insectary bioassays on the first version of PermaNet and on Dawa took place in Pakistan; insectary bioassays on PermaNet 2.0 took place at the London School of Hygiene and Tropical medicine (LSHTM). Chemical assays of residual deltamethrin also took place at LSHTM.

8.2.2 Nets and treatments

For each field trial, the bet nets used for conventional treatment had the following material specifications.

Pakistan trials: green polyester multifilament, 100 denier, mesh 156 holes/inch², dimensions 220cm long x 150cm high x 180cm wide, manufactured by SiamDutch Mosquito Netting Co., Bangkok, Thailand.

Iran trial: white polyester multifilament, 100 denier, mesh 156 holes/inch², dimensions 180cm long x 150cm high x 130cm wide, manufactured by Vestergaard Frandsen Disease Control Textiles, Hanoi, Vietnam.

Tanzania trials 1 and 3: green polyester multifilament, 75 denier, mesh 144 holes/inch²; dimensions 180cm long x 150cm high x 130cm wide, manufactured by A to Z Textiles, Arusha, Tanzania. For the 2nd Tanzanian trial, nets were the same as for the Iran trial.

Standard procedures were followed for the mixing of pyrethroid insecticide treatments, and the dipping and drying of nets (Chavasse *et al.*, 1999). In Pakistan conventionally treated deltamethrin nets (CTDNs) were impregnated with dilute K-Othrin® 2.5% SC (AgrEvo, Berkhamsted, U.K.) to give a target dosage of deltamethrin 50 mg ai/m² (approximating the PermaNet dose) for the first trial and 25mg ai/m² for the second trial, in which it was decided to compare PermaNet to a standard dosed CTDN. For the Iranian and 2nd Tanzanian trials, CTDNs were treated with aqueous suspensions of KO-tab® (Aventis, Centurion, South Africa), a pre-packaged individual net treatment which gives a deltamethrin target dose of 25mg ai/m² (WHO, 1999). Conventionally treated alphacypermethrin nets were impregnated with dilute Fendona® 10% SC (WHO, 1998a) giving a target dosage of 15mg ai/m² for trials in Pakistan (supplied by BASF, Mount Olive, NJ, USA) and 20mg ai/m² for trials in Tanzania (insecticide supplied by America Cyanamid, Gembloux, Belgium).

PermaNet is made of polyester multifilament (denier 100, mesh 156 holes/inch²), surface-treated with deltamethrin at 45-55 mg ai/m² in a resin foundation (WHO, 2004a). Originally made in white or green, PermaNet of both colours were included

in the 1st Tanzanian trial but only white PermaNets were evaluated in other trials. Samples of the earlier version of PermaNet were supplied by Vestergaard Frandsen as follows: early 2000 to Tanzania for trial 1, February 2001 to London and forwarded to Pakistan and Tanzania for the first Pakistan trial and the second Tanzania trial, April 2001 to Iran, April 2002 to Tanzania for trial 3. Samples of PermaNet 2.0 were received in April 2003 in Pakistan.

Dawa net is made to the same specifications as the nets used for conventional treatment (above) but is pre-treated with deltamethrin by the manufacturer purportedly using a similar method to PermaNet™, the insecticide being bound in a resin to the surface of the polyester fibres giving a target dose of 50mg ai/m².

8.2.3 *Washing procedures*

In 5 of the 6 PermaNet trials the nets were washed vigorously by hand for 2-3 min in tap-water at ambient temperature, according to local practices. In Iran the water was chlorinated, but not in Pakistan or Tanzania. The soaps and detergents used were: Lux™ soap bar (www.unilever.com) 4 g/L giving pH 7.9 solution in Pakistan; Barf™ powder (Paksun Co., Tehran) detergent in Iran; local soap and Foma™ (SDL, Tanga, Tanzania) detergent 1.8 g/L giving pH 9.75 solution in Tanzania.

In the second Pakistan PermaNet trial, and in the Pakistan trial of Dawa net the washing methods preceding insectary bioassays and overnight platform tests differed from other trials. Intact bed nets destined for overnight platform testing were subjected to a harsher wash regime, the nets being soaked for 60 min in water at ambient temperature, washed for 30 min in a top-loading 2-way-spin washing machine with water at 40 ± 5°C and rinsed for 15 min at ambient temperature in the washing machine. Swatches of net tested in insectary bioassays were washed using the newly-established interim standard wash practice of WHO (2004a). Swatches of net (25cm x 25cm) washed in 0.5L of alkaline (pH 9) soap solution (2 g/L) in deionised water at 30°C in a water bath shaker (model SW 20: Julabo GmbH, Seelbach, Germany) at 155 strokes/min for 10 min, followed by two 10 min rinses in deionised water at 30°C, also using the water bath shaker. All nets and net swatches were dried in the shade. One set of net swatches was stored before washing and the other sets after 10, 20 and 30 washes. These were examined for deltamethrin content using HPLC, and the PermaNet 2.0 swatches tested by insectary bioassay in London.

8.2.4 Test methods

The test methods used in the seven trials are shown in Table 8-1 below.

Insectary bioassays

Insectary-reared susceptible *Anopheles stephensi* were used for bioassays in Pakistan and London, whereas wild-caught mosquitoes were used elsewhere: *An. stephensi* in Iran, *An. gambiae* Giles and *An. funestus* Giles in Tanzania.

Both fixed-time exposure and median time to knockdown contact bioassays were performed (Chapter 2, section 2.2). In bioassays carried out in Pakistan or London, WHO plastic bioassay cones were used to expose mosquitoes to netting in 3 min exposure tests, and the method of confining mosquitoes in a wire ball-frame with netting wrapped around was used to determine MTKD. In all other trials the wire ball-frame exposure method was used for all types of bioassay.

Table 8-1 Test methods used in seven trials of candidate LLIN

Trial	Insectary bioassay method:		Overnight field test method	HPLC assay of deltamethrin content
	% mortality 24 h after 3 min exposure	Median time to knockdown (MTKD)		
1 st Tanzania PermaNet trial*	Yes	Yes	Not done	No
2 nd Tanzania PermaNet trial*	Yes	Yes	Experimental hut	No
3 rd Tanzania PermaNet trial*	No	Yes	Experimental hut	No
Iran PermaNet trial*	Yes	Yes	Not done	No
1 st Pakistan PermaNet trial	Yes	Yes	Enclosed platform	No
2 nd Pakistan PermaNet trial (PermaNet 2.0)	Yes	No	Enclosed platform	Yes
Pakistan Dawa trial	Yes	No	Enclosed platform	Yes

* These trials were carried out by separate research groups and the author was not involved in the study design or data collection.

Trials of nets in experimental huts and on enclosed platforms

In Tanzania, field trials were carried out in experimental huts fitted with veranda-traps (Smith & Webley, 1969; Curtis *et al.*, 1996). Inside each hut, two volunteers slept overnight within the test net. Host-seeking wild mosquitoes (anophelines and culicines) were able to enter and exit the hut via open eaves on two sides. Eaves on the other two sides opened into screened verandas which trapped the mosquitoes exiting on those sides; there were also exit traps on the hut windows. For each hut, the numbers of mosquitoes dead and blood-fed were recorded after sunrise, and the total number of mosquitoes entering the hut, T_e , was estimated as $T_h + T_w + 2 T_v$, where T_h = number of mosquitoes found in the hut, T_w = number of mosquitoes in the window traps and T_v = number of mosquitoes in the two screened veranda traps (the other two verandas being open).

In the Pakistan trials the overnight platform tests were carried out using the standard method described in Chapter 2, section 2.2. Two sleepers lay under each test net. To assess mosquito blood-feeding inhibition by the net treatment in the second trial the nets were deliberately holed (six 4x4cm holes) to simulate worn and torn nets.

The overnight platform tests of unwashed and washed nets of PermaNet 2.0, Dawa net, CTDN and untreated nets were tested simultaneously. The PermaNet and Dawa results are presented separately (Table 8-8 and 8-11) in order to show the statistical comparison of PermaNet with CTDN and untreated nets and, separately, Dawa with CTDN and untreated nets (the data for the CTDN and the untreated nets in these two tables are therefore identical).

Assay of deltamethrin using high pressure liquid chromatography (HPLC)

Nets assayed were from the second Pakistan PermaNet trial and the Pakistan Dawa trial, so PermaNet 2.0, CTDN and Dawa, that had been washed (by the washing machine method) and used for overnight platform tests, plus swatches of net that had been washed (by the water bath method). These were tested at the London School of Hygiene and Tropical Medicine (LSHTM) for deltamethrin content by HPLC, using the Dionex Summit (Camberley, Surrey, UK) range of equipment and software. Deltamethrin was extracted with acetonitrile and samples were separated on an Acclaim^R C₁₈ 120Å (250 X 4.6 mm column) by eluting with water/acetonitrile (90:10% v/v) at a flow rate of 2 ml/min and passed through the photodiode array detector (PDA-100, Dionex) set at 275 nm. Authenticity of

detected peaks was determined by comparison of retention time, spectral extraction at 275nm and spiking the sample with a commercially available standard deltamethrin. A calibration curve of deltamethrin was generated by Chromeleon software (Dionex) using known amounts of the standard (0.0 – 0.4 ug/ml) in acetonitrile, then used to estimate the amount of deltamethrin in the net samples (four replicates for each treatment type and wash number). These work was done by the LSHTM HPLC technician with assistance from the author.

8.2.5 Statistical analyses

The data from the first Tanzania trial was collected and analysed by another LSHTM research team (which did not include the author) and are presented here for comparison. The raw data from the second and third Tanzania trials and the Iran trial were kindly supplied by those research teams (in Excel 98 databases). These were analysed by the author along with the author's own data from the Pakistan trials.

In the first Tanzanian trial, nets were bioassayed after each wash (3 nets), or every 4th wash (8 nets). Linear regressions of mortality and MTKD on the number of washes were calculated for each type of net: i.e. white or green PermaNet or net conventionally treated with alphacypermethrin. If significant, the regression coefficients were used to predict the mortality or MTKD values after 20 washes. In the other five trials on PermaNet, bioassays were done after a variety of wash cycles standard within each trial. In these, mean, median times to knockdown after washing on each LLIN were compared to that on the CTDN using t-tests. Proportional data on mosquitoes (i.e. proportions knocked-down, dead or blood-fed) were analysed using blocked logistic regression. Comparisons between treatments were made by successively dropping treatments from the overall comparison. This process allowed each treatment to be compared with every other. Means and confidence limits of the constant for each treatment were back-transformed for presentation, as follows:

$$x' = \frac{1}{1 + (1/\exp(x))} \quad \text{where: } x' = \text{back-transformed value} \\ x = \text{the value from the logistic regression}$$

Total numbers of mosquitoes caught in the experimental huts, each night for each treatment, were compared using the Scheffe multiple comparison test to investigate any differences in repellency between the treatments. In the Pakistani trials, where

enclosed platforms were used, repellency could not be measured as mosquitoes were introduced into an enclosed area from which they could not escape.

All analyses were carried out using STATA 6.

8.3 Results

8.3.1 First version of PermaNet

Fixed-time contact bioassays *Anopheles* mortality after contact with unwashed treated nets (the original version of PermaNet and two types of CTDN) was consistently 100% in the Iran trial and all Tanzanian studies (Tables 8-2, 8-3). However, in the 1st Pakistan PermaNet trial baseline mortality was much lower after contact with unwashed treated nets, but was significantly more for PermaNet (94.2%) than for CTDNs (85.2% for deltamethrin SC 50mg ai/m² and 69.1% for alphacypermethrin SC 15 mg ai/m²) (Table 8-3).

After repeated washing (the number of wash cycles ranged from 8 to 21 in different trials), bioassays on white PermaNets gave 97.7-100% mortality (Table 8-3) and hence showed no significant loss of insecticidal efficacy, whereas washed CTDNs showed significantly lower mortality rates: 70% in Pakistan (21 washes of net with 50 mg deltamethrin/m² from K-Othrin), 64.8% in Iran and 81.8% in Tanzania 2nd trial (respectively 15 and 12 washes of nets with 25mg deltamethrin/m² from KO-tab). Nets conventionally treated with alphacypermethrin also showed significantly reduced efficacy after washing: mortality ranged from 40.9-95.5% on nets treated with 20mg ai/m² after 8–21 washes in Tanzania (Table 8-2) and 49.1% on nets treated with 15mg ai/m² after 21 washes in Pakistan (Table 8-3).

The loss of insecticidal efficacy after washing of the green PermaNets tested in the 1st Tanzanian trial was no different from that on alphacypermethrin treated nets (Table 8-2); results between individual nets were, however, not consistent.

Median time to knockdown bioassays. The median time to knockdown (MTKD) on unwashed white PermaNet (first version) was around 500s in all five trials and was similar to that on unwashed CTDNs in the 1st Pakistan and Iran trials (Table 8-4). In the latter study, the MTKD after 15 washes increased 1.7-fold on CTDN ($P<0.001$) but did not change significantly on PermaNet ($P=0.42$). In the 2nd Tanzania trial (Table 8-4), the MTKD after 12 washes rose 2.5-fold on CTDN ($P<0.001$) but, as in the Iranian study, showed no significant increase on PermaNet ($P=0.262$).

By contrast, in the 1st Pakistan study the MTKD after 21 washes doubled on both PermaNet ($P<0.001$) and CTDN ($P<0.001$) (Table 8-4). In the 3rd Tanzania study the MTKD after 15 washes almost doubled on PermaNet ($P<0.001$) and rose 2.3-fold for nets treated conventionally with 20mg alphacypermethrin/m² (Table 8-4). Moreover, in the 1st Tanzania trial (Table 8-2) the mean MTKD doubled for white PermaNets after 9 to 20 washes ($P<0.001$) and almost tripled for green PermaNets washed 20 to 21 times ($P=0.002$), compared with increases of 1.6–3.5-fold for conventionally treated alphacypermethrin nets washed 8 to 21 times ($P<0.001$).

Thus by the MTKD criterion, the first version of PermaNet showed inconsistent wash-resistance, while outperforming the comparison ITNs in three of the five trials which included MTKD tests.

Overnight platform tests with whole nets. Anopheline mosquito mortality rates were far less during the enclosed platform trials in Pakistan (Table 8-5) than in the experimental huts in Tanzania (Table 8-6). Conversely, wild culicine mortality was far less in Tanzanian huts than in the Pakistan test setting (c.f. Tables 8-5 & 8-6). In Pakistan culicine mortality was similar to anopheline mortality rates (Table 8-5). Low culicine mortality has consistently been observed at this Tanzanian study site (Curtis *et al.* 1996) and is probably due to pyrethroid resistance (Khairandish & Wood, 1993).

Culicine mortality was similar to anopheline mortality rates (Table 8-5). Predominant species involved were *Anopheles gambiae*, *An. funestus* and *Culex quinquefasciatus* in Tanzania (Curtis *et al.*, 1996); *An. stephensi*, *An. subpictus*, *An. nigerrimus*, *An. pulcherrimus*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. bitaeniorhynchus* and *Cx. vishnui* in Pakistan (Graham *et al.*, 2002a,b).

The first trial in Pakistan evaluated bed nets on enclosed platforms for 3 months (Table 8-5), comparing the first version of PermaNet versus untreated polyester net and a net conventionally treated with deltamethrin at 50 mg ai/m² (CTDN). Figure 8-1 shows the insecticidal efficacy of these nets after 0, 5, 10 and 15 washes. For both types of treated net, the 24 hour mortality rate rose slightly at 5 washes and then declined at 10 and 15 washes to below the unwashed starting level, this trend being shown also by mortality rates on the untreated (control) net, reflecting seasonal fluctuations in ambient conditions with temperature and humidity being higher during the tests at 5 washes (August) than at 10 washes (September) or 15 washes (October). After correction by Abbott's formula to adjust for control mortality, the performance after washing declined less than indicated by the unadjusted data,

with no significant difference between the PermaNet and CTDN. After 15 washes, the treatment-induced mortality of *An. stephensi* was reduced from 35% to 20% for the washed CTDN and from 36% to 15% for the washed PermaNet, while an unwashed PermaNet tested in parallel gave only 20% mortality at the end-point (Figure 8-1). For both types of treated net in the 1st Pakistan trial, culicine mortality rates of ~16-18% did not decline significantly with washing (Table 8-5).

In the second Tanzanian trial (Table 8-6) comparing PermaNet (first version) with CTDN (KO-tab 25mg ai/m²), *Anopheles* mortality on PermaNet remained high (>90%) after 15 washes, but appeared to fall on CTDN, from 84% to 70% after 15 washes, although this decline was not significant ($P=0.162$). Culicine mortality rates were far lower on treated nets (3.3–6.7%) and not significantly different between net types, irrespective of washing. Both types of treated net stopped the majority of mosquitoes from biting successfully. Blood-feeding rates of anophelines showed no change after 15 washes of the nets, whereas culicine blood-feeding rates appeared to increase (Table 8-6), although this trend was not significant.

Similarly in the third Tanzanian trial, 24 hour mortality rates of anophelines (86-94%) and culicines (0.4-1.2%) were apparently unaffected after 15 washes of PermaNet (first version) and CTDN (KO-tab), both of which prevented the majority of mosquitoes from blood-feeding (Table 8-6).

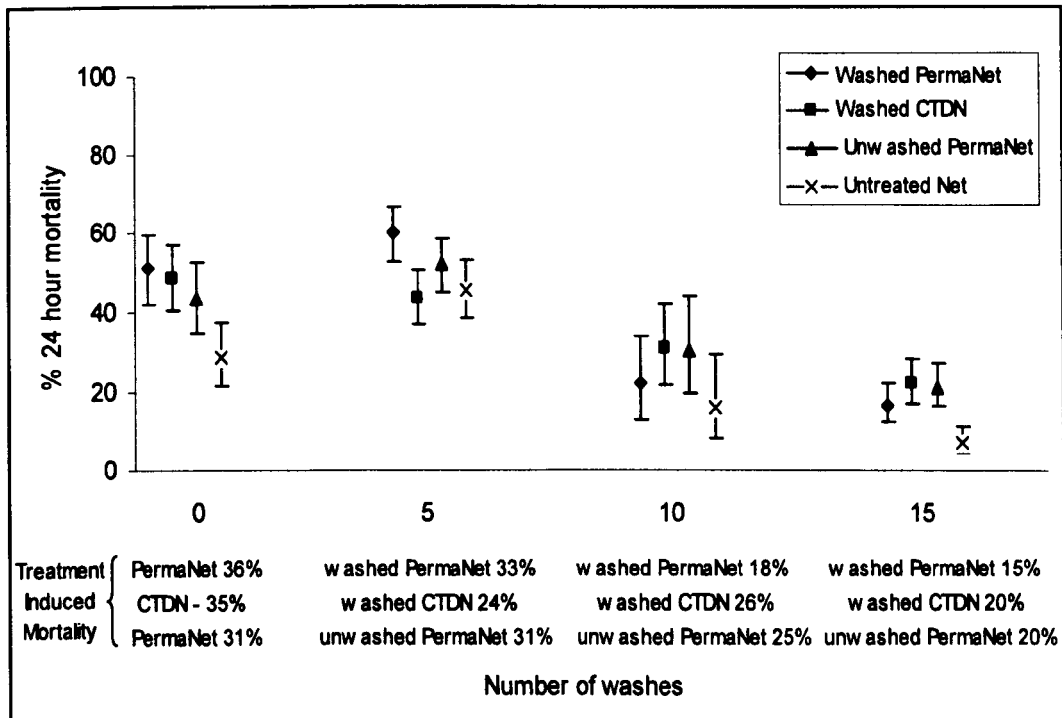


Figure 8-1. Overnight platform tests in the first Pakistan PermaNet trial: *Anopheles stephensi* % mortality.

Notes:

1. Data points for each number of washes are the mean of 15 successive nights (replicates): each type of unwashed net was first tested on 5 platforms in rotation for 15 nights; after washing up to 5 times they were then tested again in rotation on 5 platforms for 15 nights; after washing up to 10 times they were then tested again in rotation on 5 platforms for 15 nights; finally after washing up to 15 times they were again tested in rotation on 5 platforms for 15 nights.
2. The CTDN was treated to a target dose of 50mg ai/m².
3. Treatment induced mortality was calculated using Abbott's formula, corrected against the mean value for the untreated net.

Table 8-2. Insectary bioassay tests in the first Tanzania PermaNet trial using wild-caught *Anopheles gambiae*. *Permission to reproduce data was granted by C. Curtis and colleagues.*

Type of net and treatment	Date of test (m/y)	Total no. washes ²	3 min exposure				Median time to knockdown (seconds)			
			Individual nets		Nets grouped for analysis		Individual nets		Nets grouped for analysis	
			% 24 h Mortality before washing	% 24 h Mortality after last wash ⁶	Regression coefficient (mortality on no. washes) ³	Predicted mortality after 20 washes	MTKD before washing (seconds)	MTKD after last wash (seconds)	Regression coefficient (MTKD on no. washes) ³	Predicted MTKD after 20 washes (seconds)
PermaNet (green)	Jul-00	¹ 21	100%	100.0%	-0.40***	93.4%	⁵ 396	⁵ 1119	+26.82**	1191
	Jul-00	¹ 21 ⁵	100%	87.9%			⁵ 401	⁵ 1424		
	Aug-00	¹ 20 ⁴	100%	90.9%			⁴ 426	⁴ 909		
PermaNet (white)	Mar-00	¹ 20 ⁴	100%	100.0%	[-0.55]	[100%]	² 407	² 538	+42.40***	1513
	Apr-00	³ 9	100%	97.7%			² 490	⁴ 1007		
	Jun-00	³ 15	100%	100.0%			⁴ 514	⁴ 1422		
Alphacypermethrin 20mg ai/m ²	Aug-00	¹ 21	100%	100.0%	-0.65**	86.9%	⁴ 494	² 772	+24.02***	1091
	Mar-01	¹ 12	100%	40.9%			² 372	² 764		
	Apr-01	¹ 20 ⁴	100%	86.4%			² 362	² 1268		
	May-01	³ 8	100%	88.6%			² 317	⁴ 786		
	Jun-01	³ 14	100%	95.5%			⁴ 449	⁴ 1247		

Notes:

1. MTKD values are the mean of 2-5 tests (indicated by superscript prefix); mortality values are the mean of 3 replicate tests (each with 11 mosquitoes).
2. The number of days between each wash and each test (1 or 3) is indicated by prefix superscript.
3. Regression coefficient significance of departure from zero indicated by: * P<0.05; ** P<0.01; *** P<0.001, or [not significant].
4. Tested after every 4 washes; all other nets tested after each wash.
5. Local soap used for washing; all other nets washed with Foma detergent.
6. Considerable fluctuations occurred between successive bioassay test results for each net: for some nets the final bioassay mortality rate was more than some preceding values.

Table 8-3. 3 minute exposure bioassays of *Anopheles* mosquitoes. *Permission to reproduce data was granted by C. Curtis and colleagues, and M. Kayedi and colleagues.*

Type of net and treatment		First Pakistan PermaNet trial: 21 washes (insectary-reared, non-blood-fed <i>An. stephensi</i>)		Second Tanzania PermaNet trial: 15 washes (wild-caught, blood-fed <i>An. gambiae</i>)		Iran PermaNet trial: 15 washes (wild-caught, blood-fed <i>An. stephensi</i>)	
		No.	% 24 h	No.	% 24 h	No.	% 24 h
		tests	mortality	tests	mortality	tests	mortality
PermaNet	Unwashed	8	94.2 ^a	8	100.0 ^a	5	100.0 ^a
	Washed	5	98.2 ^a	8	100.0 ^a	10	97.7 ^b
Deltamethrin SC 50mg ai/m ²	Unwashed	8	85.2 ^b	-	-	-	-
	Washed	5	70.0 ^c	-	-	-	-
KO-tab net	Unwashed	-	-	8	100.0 ^a	5	100.0 ^a
	Washed	-	-	8	81.8 ^b	10	64.8 ^c
Alphacypermethrin SC 15mg ai/m ²	Unwashed	8	69.1 ^c	-	-	-	-
	Washed	5	49.1 ^d	-	-	-	-
Untreated Net		8	0 ^e	-	-	5	0 ^d

Note:

1. Percentage mortality and 95% CIs are back-transformed from values calculated by the blocked logistic regression model.
2. Within each column rows not sharing a superscript letter significantly by blocked logistic regression ($P < 0.05$).

Table 8-4. Insectary bioassays tests in Pakistan, Tanzania and Iran. *Permission to reproduce data was granted by C. Curtis and colleagues, and M. Kayedi and colleagues.*

Type of net and treatment		First Pakistan PermaNet trial: 21 washes (insectary-reared, non- blood-fed <i>An. stephensi</i>)		Second Tanzania PermaNet trial: 12 washes (wild-caught, blood-fed <i>An. gambiae</i>)		Third Tanzania PermaNet trial: 15 washes (wild-caught, blood-fed <i>An. funestus</i>)		Iran PermaNet trial: 15 washes (wild-caught, blood-fed <i>An. stephensi</i>)	
		No. tests	Mean MTKD (95% CI)	No. tests	Mean MTKD (95% CI)	No. tests	Mean MTKD (95% CI)	No. tests	Mean MTKD (95% CI)
PermaNet	Unwashed	8	549 (401-690) ^a	8	572 (482-662) ^a	22	434 (403-464) ^a	5	444 (250-704) ^a
	Washed	5	1045 (934-1155) ^b	8	633 (547-720) ^a	20	822 (719-925) ^b	5	526 (417-656) ^a
Deltamethrin SC 50mg ai/m ²	Unwashed	8	456 (337-575) ^a	-	-	-	-	-	-
	Washed	5	1019 (879-1158) ^b	-	-	-	-	-	-
KO-tab net	Unwashed	-	-	8	396 (352-439) ^b	-	-	10	499 (273-808) ^a
	Washed	-	-	8	983 (765-1200) ^c	-	-	10	858 (656-1048) ^b
Alphacypermethrin SC 15 or 20mg ai/m ²	Unwashed	8	523 (430-615) ^a	-	-	14	420 (374-465) ^a	-	-
	Washed	5	999 (872-1127) ^b	-	-	10	964 (727-1201) ^b	-	-
Untreated Net		8	no knockdown	8	no knockdown	-	-	-	-

Notes:

1. Within each column, rows not sharing a superscript letter differ significantly by blocked logistic regression ($P < 0.05$).
2. Target dosages of alphacypermethrin were 15mg ai/m² in the Pakistan trial and 20mg ai/m² in the 3rd Tanzania trial.

Table 8-5. Overnight platform tests in the first Pakistan PermaNet trial

Type of net and treatment		No. of nights	No. of mosquitoes per platform per night Mean (SD)	% 24 h mortality (95% CI)
<i>An. stephensi</i>				
PermaNet	Unwashed	15	15.7 (9.2)	21.2 ^a (16.4 – 26.9)
	Washed 15x	15	15.4 (10.7)	16.5 ^a (12.2 – 21.8)
CTDN 50 mg ai/m ²	Washed 15x	15	13.4 (8.3)	21.9 ^a (16.7 – 28.1)
Untreated Net		15	16.0 (13.1)	7.1 ^b (4.4 – 11.1)
Culicines				
PermaNet	Unwashed	15	375.2 (104.8)	16.2 ^b (15.2 – 17.2)
	Washed 15x	15	390.3 (144.3)	17.7 ^a (16.8 – 18.7)
CTDN 50 mg ai/m ²	Washed 15x	15	382.6 (98.6)	17.5 ^{ab} (16.5 – 18.5)
Untreated Net		15	376.4 (94.8)	7.2 ^c (6.6 – 7.9)

Notes:

1. Percentage mortality and 95% CIs were back-transformed from values calculated by the blocked logistic regression model.
2. Within each column and genera, values not sharing a superscript letter, differ significantly ($P < 0.05$) by blocked logistic regression.
3. Numbers of mosquitoes per night are not indicative of relative repellence or attractiveness of the net on each platform; wild caught, host-seeking mosquitoes were manually introduced to the enclosed nets and were unable to leave the platform.

Table 8-6. Experimental hut tests in the second and third Tanzania PermaNet trials. *Permission to reproduce data was granted by C. Curtis and colleagues, and M. Kayedi and colleagues.*

Type of net and treatment		Second Tanzania PermaNet Trial				Third Tanzania PermaNet Trial			
		No. of nights	Mean no. of mosquitoes per hut per night	% blood-fed	% mortality	No. of nights	Mean no. of mosquitoes per hut per night	% blood-fed	% mortality
Anophelines									
PermaNet	Unwashed	15	6.2 ^a	7.0 ^a	97.7 ^b	14	5.4 ^a	6.6 ^a	85.5 ^a
	Washed 15x	13	6.4 ^a	3.6 ^a	91.6 ^b	21	5.3 ^a	1.8 ^a	93.8 ^a
KO-tab net	Unwashed	13	2.4 ^a	9.7 ^a	83.9 ^{ab}	-	-	-	-
	Washed 15x	14	4.1 ^a	8.8 ^a	70.2 ^a	21	4.0 ^a	4.8 ^a	88.0 ^a
Untreated Net		13	6.0 ^a	32.1 ^b	25.6 ^c	16	3.4 ^a	56.4	34.6 ^b
Culicines									
PermaNet	Unwashed	15	9.7 ^a	2.1 ^c	3.5 ^a	14	36.6 ^a	4.3 ^a	1.2 ^a
	Washed 15x	13	13.8 ^a	5.0 ^{abc}	6.7 ^a	21	34.6 ^a	5.9 ^a	0.4 ^a
KO-tab net	Unwashed	13	9.1 ^a	5.1 ^{abc}	5.1 ^a	-	-	-	-
	Washed 15x	14	10.8 ^a	10.6 ^b	3.3 ^a	21	27.5 ^a	3.8 ^a	0.5 ^a
Untreated Net		13	7.3 ^a	9.5 ^{ab}	0 ^b	16	44.1 ^a	31.3	0 ^b

Notes:

1. Percentage mortality and blood-fed are back-transformed from values calculated by the blocked logistic regression model.
2. Within each column and genera, values not sharing a superscript letter differ significantly (P<0.05).
3. Anophelines were nearly all *An. gambiae*, plus a few *An. funestus* in trial 3.

8.3.2 PermaNet 2.0

Fixed-time contact bioassays. Before washing, there were no significant differences in mortality or knockdown between PermaNet 2.0 and CTDN (Table 8-7). After 10 washes of PermaNet 2.0, there was no significant reduction in mortality compared to unwashed PermaNet 2.0 ($P=0.2$). After 20 washes of PermaNet 2.0, bioassays still gave approximately 80% knockdown and mortality although both these were significantly lower than on unwashed PermaNet 2.0 (knockdown $P<0.001$; mortality $P=0.039$). After 30 washes both knockdown and mortality on PermaNet 2.0 had declined to suboptimal levels. In contrast to the swatches of LLIN, swatches of CTDN showed a dramatic reduction in efficacy after 10 water-bath washes, knockdown and mortality both being very low ($P<0.001$ for both); whilst a small proportion of mosquitoes were knocked down 1 hour after exposure to the 20 or 30 times washed CTDN these mosquitoes recovered during the 24 hour holding period resulting in no mortality.

Overnight platform tests. Mosquito mortality rates were generally low during the 2nd Pakistan PermaNet trial and showed little variation: anophelines 14.1–21.6%, culicines 7.8–16.5% (Table 8-8). On the CTDN (50 mg ai/m²), mortality was significantly lower after exposure to treated nets washed 20 times than on the unwashed nets, for culicines ($P<0.001$) and anophelines ($P=0.032$), but not when washed 10 times only. By contrast, there was no decline in mortality on PermaNet 2.0 after 20 washes, for culicines ($P=0.636$) or anophelines ($P=0.993$). With the untreated net, the proportion of culicines (4.3%) and anophelines (9.0%) blood-feeding (Table 8-8) was too low for detection of any treatment-induced effects.

Chemical assay of deltamethrin concentration on nets. Deltamethrin content on unwashed whole PermaNet 2.0 was found to be very variable (27 – 142 mg ai/m²), although the mean value of 55.3 (95% CI: 10.1 – 100.6) mg ai/m² came very close to the target concentration. Generally, the HPLC assay (Table 8-9) showed low variability in deltamethrin content between samples at each wash point.

Swatches of PermaNet 2.0 had a high deltamethrin content before washing (mean 86.3, range 83 – 92 mg ai/m²) which decreased by almost half over the first 10 washes, by another ~50% over the next 10 washes, and by 75% between 20 and 30 washes. On average, the first 10 washes removed 37 mg ai/m² and the final 10 washes removed 18 mg/m² of insecticide. A similar rate of removal was observed for machine-washed PermaNet 2.0; deltamethrin content fell by ~50% after 10 washes and by a further 50% after 20 washes.

Deltamethrin concentrations remaining on PermaNet 2.0 after 20 washes in the water bath (24.1 mg ai/m²) or washing machine (13.1 mg ai/m²) were similar to the range normally recommended for newly treated conventionally treated nets (WHO, 1999). However, this impressive amount withstanding 20 washes appears to have been at least partly due to the high loading dose of deltamethrin at the point of manufacture.

Before washing, the insecticide dosage was found to be higher on all the treated net swatches destined for water bath washing than on the equivalent whole nets destined for machine washing (Table 8-9). This may have been the result of whole nets being handled far more than the net swatches; the whole nets (unwashed and washed) had been field-tested on enclosed platforms before HPLC assay and therefore might have lost some insecticide by weathering and abrasion during use.

These two methods of washing readily stripped deltamethrin from the CTDNs so that very low amounts were detectable after 10 - 20 washes. On 16 swatches of CTDN, with one exception, no deltamethrin was detected after washing 10 or 20 times (anomalous detection of 29 mg ai/m² on one piece after 20 washes may indicate uneven stripping or contamination). The insectary bioassays on these net swatches suggest that levels of insecticide too low to be detected by HPLC may still cause temporary knockdown, though not mortality: a small number of mosquitoes were knocked down 1h after exposure to the 10 and 20 times washed swatches of CTDN, but recovered during the 24 hour holding period.

Table 8-7. Fixed-time exposure bioassays in the second Pakistan PermaNet trial using *Anopheles stephensi*. Swatches of net were washed using the WHOPES water bath method and tested in LSHTM.

Treatment	0 washes	10 washed	20 washes	30 washes
% mortality, (95% CI)				
PermaNet 2.0	97.7% (85.3 - 99.7)	90.7% (77.7 - 96.5)	81.8% (67.7 - 90.6)	43.2% (29.5 - 58.0)
CTDN 25mg ai/m ²	90.9% (78.2 - 96.5)	14.0% (6.4 - 27.8)	0.0% (0 - 0)	0.0% (0 - 0)
% knockdown, (95% CI)				
PermaNet 2.0	100% (100-100)	97.7% (85.3 - 99.7)	79.5% (65.1 - 89.0)	77.3% (62.7 - 87.3)
CTDN 25mg ai/m ²	93.2% (80.9 - 97.8)	46.5% (32.3 - 61.3)	11.4% (4.8 - 24.5)	2.3% (0.3 - 14.4)

Notes:

1. Means are of 4 replicate tests with batches of 10 or 11 mosquitoes at each treatment and time point.
2. Percentage mortality, knockdown and 95% CIs are back-transformed from values calculated by the blocked logistic regression model.
3. Net swatches tested are those subsequently tested by HPLC analysis (table 8-8).
4. Mortality in tests carried out on untreated nets as negative controls was never more than 10%.

Table 8-8. Overnight platform tests in the second Pakistan PermaNet trial.

Treatment		No. of nights	No. of mosquitoes per platform per night Mean (SD)	Mortality % (95% CI)	Blood-fed % (95% CI)
Anophelines					
CTDN 25mg ai/m ²	Unwashed	20	36 (20)	18.3% ^{ab} (15.6 - 21.3)	12.4% ^{ab} (10.2 - 15.0)
	Washed 10x	20	38 (22)	21.1% ^a (18.3 - 24.1)	7.8% ^c (6.1 - 9.9)
	Washed 20x	21	33 (17)	14.1% ^c (11.7 - 16.9)	12.2% ^a (9.9 - 14.8)
PermaNet 2.0	Unwashed	21	38 (23)	20.6% ^a (17.9 - 23.5)	12.9% ^{ab} (10.7 - 15.4)
	Washed 10x	21	31(17)	21.6% ^a (18.6 - 25.0)	11.0% ^{ad} (8.8 - 13.7)
	Washed 20x	21	30 (21)	20.5% ^a (17.6 - 23.9)	16.1% ^b (13.5 - 19.2)
Untreated net		20	36 (18)	16.1% ^{bc} (13.6 - 19.0)	9.0% ^{cd} (7.1 - 11.3)
Culicines					
CTDN 25mg ai/m ²	Unwashed	20	333 (119)	11.0% ^a (10.3 - 11.8)	4.8% ^a (4.3 - 5.3)
	Washed 10x	20	361 (131)	11.9% ^a (11.2 - 12.7)	1.0% ^b (0.8 - 1.2)
	Washed 20x	21	353 (127)	9.0% ^b (8.4 - 9.7)	3.6% ^c (3.2 - 4.0)
PermaNet 2.0	Unwashed	21	373 (131)	13.6% ^c (12.9 - 14.4)	1.6% ^d (1.3 - 1.9)
	Washed 10x	21	364 (122)	16.5% ^d (15.7 - 17.3)	1.5% ^d (1.3 - 1.8)
	Washed 20x	21	369 (147)	13.9% ^c (13.1 - 14.7)	1.8% ^d (1.5 - 2.1)
Untreated net		20	403 (134)	7.8% ^e (7.2 - 8.4)	4.3% ^a (3.9 - 4.8)

Notes:

1. Percentage mortality, blood-fed and 95% CIs are back-transformed from values calculated by the blocked logistic regression model.
2. Within columns, values not sharing a superscript letter differ significantly ($P < 0.05$).
3. Numbers of mosquitoes per night do not provide information about the repellence or attractiveness of treatments, since wild-caught mosquitoes were introduced manually and were unable to leave the trap nets over the platforms.

Table 8-9. Deltamethrin content of netting from the second Pakistan PermaNet trial and the Pakistan Dawa trial as measured by HPLC. Eight pieces of mesh were tested for each treatment type and wash interval.

	Mean deltamethrin content mg ai/m ² (95%CI) [range]			
	0 washes	10 washes	20 washes	30 washes
PermaNet washed as net swatches in water bath	86.3 (83.9 - 88.7) [83.12 - 91.68]	49.5 (47.4 - 51.5) [46.0 - 53.0]	24.1 (20.7 - 27.4) [16.4 - 29.8]	5.8 (4.3 - 7.4) [2.8 - 8.1]
Dawa washed as net swatches in water bath	149.6 (18.7 - 280.4) [24.4 - 389.9]	7.4 (-4.0 - 18.7) [0.9 - 40.5]	1.1* (0.9 - 1.2) [0.8 - 1.3]	tests not done
CTDN (25mg ai/m ²) washed as net swatches in water bath	22.9 (19.9 - 25.8) [18.0 - 27.48]	not detected	3.6** (-4.9 - 12.1) [0 - 28.8]	tests not done
PermaNet washed as whole net in machine	55.3 (10.1 - 100.6) [27.1 - 142.0]	22.8 (18.0 - 27.6) [20.0 - 26.0]	13.1 (11.2 - 14.9) [10.8 - 15.2]	tests not done
Dawa washed as whole net in machine	44.1 (12.8 - 75.3) [21.6 - 98.4]	19.6 (-10.4 - 49.6) [3.2 - 38.4]	8.3 (-1.7 - 18.3) [1.6 - 20.8]	tests not done
CTDN (25mg ai/m ²) washed as whole net in machine	11.9 (9.0 - 14.9) [8.0 - 15.2]	1.5 (0.9 - 2.0) [0.8 - 2.0]	1.4*** (1.0 - 1.8) [0 - 1.6]	tests not done

Notes:

* Not detected in 1 of 8 samples

** Not detected in 7 of 8 samples

*** Not detected in 2 of 2 samples

8.3.3 Dawa net

Fixed-time contact bioassays (3 min exposure). At zero washes Dawa and CTDN were similar with respect to both mortality and knockdown (Table 8-10). Mosquito knockdown 1 hour after exposure remained high throughout the 15 washes, dropping only to ~80% over the 15 wash period for both the CTDN and Dawa net.

Mosquito mortality after the 24 hour holding period showed a steeper decline over the 15 washes. At 3 washes there was little change. At 6 washes there was considerable recovery of knocked down mosquitoes during the 1h holding period. But despite this, mortality at 24 hours was ~50% on the CTDN and 60% on the Dawa. Mortality was slightly higher on the nets washed 10 times but declined again on the nets washed 15 times. Dawa achieved higher levels of mortality than the CTDN throughout the series but the differences were small and both nets performed poorly after 15 washes (<40% mortality).

Overnight platform tests. Mosquito mortality rates were generally low during this trial and showed little variation: anophelines 14.1–24.5%, culicines 7.8–15.0% (Table 8-11). As described above (the data for CTDN were presented before in section 8.3.2, the second Pakistani trial of PermaNet 2.0) mortality after exposure to the CTDN (50 mg ai/m²), was significantly lower on nets washed 20 times than on unwashed nets, both for culicines ($P<0.001$) and anophelines ($P=0.032$), but not at 10 washes. For Dawa, mortality on exposure to the 20 times washed net was lower than on the unwashed net, both for culicines ($P=0.007$) and anophelines ($P=0.023$), and again there was no difference on the nets washed 10 times. More culicine mosquitoes fed (9.0%) than anopheline mosquitoes (4.3%), the proportion feeding was low for both genera. The Dawa net appeared to reduce culicine blood-feeding slightly both before and after washing ($P<0.001$ for each washed Dawa net compared to the unwashed Dawa net). No effect of blood-feeding was discernable for anophelines, possibly because the proportion feeding was so low for all treatments, including the untreated nets.

Chemical assay of deltamethrin concentration on nets. Deltamethrin concentration on unwashed Dawa was found to be very variable. Pieces from the insectary bioassay tests ranged from 24 – 390 mg ai/m² and sections of whole nets from the overnight platform tests ranged from 2-98 mg ai/m² (Table 8-9). The mean value on the whole nets 44mg ai/m² (95% CI: 12.8 – 75.3) was close to the target concentration of 50mg ai/m² but the mean value of the net pieces was almost triple the target dose.

The water bath washing of net pieces appeared to strip insecticide more readily than the washing of whole nets in washing machines, both for Dawa and CTDN. After just 10 washes of this method the Dawa net was shown to contain a mean of 7.4 mg ai/m² although there was also fairly high variability at this point, perhaps a result of the very variable loading doses. No deltamethrin was detected in the CTDN after 10 washes. After 20 washes the insecticide had been almost entirely stripped from all the Dawa net pieces, regardless of loading dose, a small amount of insecticide was detected in only 1 of the 8 CTDN samples tested after 20 washes.

Insecticide was also rapidly stripped from Dawa nets washed as whole nets in the washing machines. The amount of insecticide decreases by over half after 10 washes and halved again over the next 10 washes. At both 10 and 20 washes there was again a wide range in insecticide detected in the 8 samples tested. The insecticide in the Dawa treatment did persist more successfully than that on the CTDN. In CTDN marginal amounts of insecticide were detected at 10 and 20 washes.

Table 8-10. Fixed-time exposure bioassays in the Pakistan Dawa trial using *Anopheles stephensi*. Swatches of Dawa and CTDN (25 mg ai/m²) were washed using the WHOPES water bath method.

	0 washes	3 washes	5 washes	10 washes	15 washes
	% mortality, (95% CI)				
Dawa	100% (100-100)	100% (100-100)	61.0% (53.8 - 67.7)	77.0% (70.7 - 82.3)	39.3% (32.9 - 46.2)
CTDN 25mg ai/m ²	97.5% (94.1 - 99.0)	97.9% (94.7 - 99.2)	51.5% (44.6 - 58.4)	62.7% (55.9 - 69.1)	27.2% (21.5 - 33.8)
	% knockdown, (95% CI)				
Dawa	93.0% (88.5 - 95.8)	98.0% (94.8 - 99.2)	97.3% (93.7 - 98.9)	87.0% (81.6 - 91.0)	81.5% (75.1 - 85.9)
CTDN 25mg ai/m ²	95.0% (90.9 - 97.3)	97.4% (94.0 - 98.9)	94.4% (90.2 - 96.9)	92.6% (88.2 - 95.5)	80.2% (74.1 - 85.1)

Notes:

1. Mortality on the two types of nets is significantly different at every wash point except 5 washes (although differences are small throughout). Knockdown on the two types of net is similar throughout.
2. Means are of 20 replicate tests with batches of 10 or 11 mosquitoes at each treatment and time point.
3. Percentage mortality, knockdown and CIs are back-transformed from values calculated by the blocked logistic regression model.
4. Net swatches tested are those subsequently tested by HPLC analysis
5. Negative control mortality (tests on untreated net) was always <7%.

Table 8-11. Overnight platform tests in the Pakistan Dawa trial: % blood-fed and % mortality 24 hours after initial exposure

			No. of nights	Mean no. of mosquitoes per platform per night	% mortality (95% CI)	% blood-fed (95% CI)
Anophelines						
CTDN 25mg ai/m ²	Unwashed	20	36 (20)	18.3% ^{ad} (15.6 - 21.3)	12.4% ^a (10.2 - 15.0)	
	Washed 10x	20	38 (22)	21.1% ^{ab} (18.3 - 24.1)	7.8% ^b (6.1 - 9.9)	
	Washed 20x	21	33 (17)	14.1% ^c (11.7 - 16.9)	12.2% ^a (9.9 - 14.8)	
Dawa	Unwashed	21	33 (14)	24.5% ^b (21.5 - 27.9)	11.0% ^{ac} (8.9 - 13.6)	
	Washed 10x	21	30 (13)	21.3% ^{ab} (18.3 - 24.7)	7.9% ^{bc} (6.0 - 10.3)	
	Washed 20x	21	35 (23)	19.5% ^{ad} (16.8 - 22.6)	13.1% ^a (10.8 - 15.7)	
Untreated net		20	36 (18)	16.1% ^{cd} (13.6 - 19.0)	9.0% ^{bc} (7.1 - 11.3)	
Culicines						
CTDN 25mg ai/m ²	Unwashed	20	333 (119)	11.0% ^a (10.3 - 11.8)	4.8% ^a (4.3 - 5.3)	
	Washed 10x	20	361 (131)	11.9% ^a (11.2 - 12.7)	1.0% ^b (0.8 - 1.2)	
	Washed 20x	21	353 (127)	9.0% ^b (8.4 - 9.7)	3.6% ^c (3.2 - 4.0)	
Dawa	Unwashed	21	359 (128)	15.0% ^c (14.2 - 15.8)	1.0% ^b (0.8 - 1.3)	
	Washed 10x	21	348 (130)	14.0% ^{cd} (13.2 - 14.8)	1.3% ^b (1.1 - 1.6)	
	Washed 20x	21	383 (114)	13.5% ^d (12.8 - 14.3)	1.3% ^b (1.1 - 1.6)	
Untreated net		20	403 (134)	7.8% ^e (7.2 - 8.4)	4.3% ^a (3.9 - 4.8)	

Notes:

1. Within columns, values not sharing a superscript letter differ significantly ($P < 0.05$).
2. Numbers of mosquitoes per night do not provide information about the repellency or attractiveness of treatments, since wild-caught mosquitoes were introduced manually and were unable to leave the trap nets over the platforms.
3. The data for mosquitoes exposed to CTDN is the same as that presented in Table 8-8, Dawa, PermaNet 2.0 and CTDN were tested alongside each other in the overnight tests, the data are presented separately for PermaNet 2.0 and Dawa for ease of interpretation.

8.4 Discussion

The five trials compared here of the first version of PermaNet gave inconsistent results on its wash-resistance compared to conventionally treated nets. Trials by other investigators (Gonzales *et al.*, 2002; Müller *et al.*, 2002; WHO, 2004a) also reported inconsistency of this product. Even so, drawing conclusions from bioassay mortality tests (with 3 min exposure) on net swatches, the original PermaNet performed better than conventionally treated nets after multiple washes (ranging from 8 to 21 washes) in all but the 1st Tanzanian trial. Whilst the efficacy of conventional ITNs (treated with alphacypermethrin or deltamethrin) did decline more with repeated washing, these treatments were by no means exhausted even after 21 washes. This finding conflicts with the widely held view that, after conventional pyrethroid impregnation by dipping (Chavasse *et al.*, 1999), ITNs lose insecticidal activity after only a few washes. Partial wash-fastness is an inherent property of alphacyano-pyrethroids on polyester (N'Guessan *et al.*, 2001).

Median time to knockdown (MTKD) bioassays have the capacity to reveal small differences in performance when the amount of bioavailable insecticide remains sufficient to produce uniformly high mortality in 3 min bioassays. The evidence from MTKD for superior wash-resistance of the earlier PermaNet was equivocal: the Iran and 2nd Tanzania trials showed slight but non-significant increase of the MTKD after washing, whereas in three other trials (Pakistan and Tanzania 1st and 3rd) the insecticidal efficacy of PermaNet declined like that of conventionally treated nets, the MTKD approximately doubling after washing across all 3 studies.

These inconsistencies within and between these five, independent studies of the first version of PermaNet suggest that this promising LLIN technology was being adversely affected by variation in quality within or between batches of nets. Contradictions between findings of Gonzales *et al.* (2002) and Müller *et al.* (2002) in their tests of the earlier PermaNet might also be explained by batch variation. The manufacturer has accepted this problem of variability in the production process (Kilian, 2004), and since this discovery the manufacturer has improved quality control and relaunched the LLIN as "PermaNet 2.0" in late 2002.

Using the interim WHO standard water bath washing protocol (section 3.3.1 in WHO, 2004a) in for insectary tests on PermaNet 2.0 this study demonstrated a far superior retention of efficacy after washing of the LLIN compared to the conventionally treated net. The chemical assay of deltamethrin content gives further evidence for a superior wash resistance of PermaNet 2.0, which

demonstrated high insecticide load at 20 washes and still detectable insecticide at 30 washes. By contrast on the CTDN deltamethrin levels on net swatches were too low to be detected after 10 washes.

Before undertaking these trials it was hypothesised that the superior performance of PermaNet 2.0 might be due to the high loading dose of deltamethrin in the LLIN (confirmed by HPLC assay here) rather than to improved wash resistance. Further investigation by Kayedi (2004) has gone some way to refuting this: a comparison in insectary bioassay of PermaNet 2.0 with a net treated conventionally with two KO-tabs (deltamethrin $\sim 50\text{mg ai/m}^2$) after washing with the water bath procedure, demonstrated that the high loading dose was not the sole cause of the superior performance.

The advantage of insectary bioassays of residual activity, such as the MTKD and 3 min exposure tests, for comparative evaluation of ITNs and LLINs, lies in their ability to reveal small differences in performance between treatments. While this is useful for assessing incremental improvements in LLIN technology, it is not known what magnitude of difference in such bioassays would translate to an effect that would be detectable at the levels of vector control or disease impact. Before an informed decision can be taken as to whether a new technology offers sufficient improvement over an existing technology to justify the costs and effort of substitution, more information than that given by simple residual bioassays is needed. Field trials (overnight platform tests or experimental hut tests) with free-flying mosquitoes, enabling observations of more natural interactions between females and the insecticidal net, provide a better indication of LLIN performance in everyday use.

Improved bioassay mortality rates achieved with some early batches of PermaNet did not necessarily translate to a detectable reduction of blood-feeding success or survival rates in field comparisons with conventionally treated nets. For example, in the (first) Pakistan enclosed platform trial and in one of the two trials in Tanzanian huts, performance of the earlier PermaNet remained equivalent, not superior, to that of conventionally treated nets even after 15 washes. Only in the second Tanzania trial did PermaNet show superior performance to CTDN at the point of 15 washes. This illustrates that the earlier batches of PermaNet were of variable quality, an inference recently acknowledged in the 7th WHOPES Working Group Report (WHO, 2004a).

On the basis of these results and two other field trials (Akogbeto, 2003; Kilian *et al.*, 2004) and one other insectary trial (Duchon *et al.*, 2003), PermaNet 2.0 obtained

WHOPES *interim recommendation* for use in prevention and control of malaria (section 3.5 in WHO, 2004a). It is important that evaluation of PermaNet 2.0 in everyday use in different cultural and epidemiological settings takes place, both to examine the effectiveness of LLIN in these various settings and to ensure that the variable quality hampering the usefulness of the first version of PermaNet has been resolved.

It appears that the Dawa net treatment may suffer from similar inconsistencies in treatment which plagued the first version of PermaNet. Certainly the few nets tested here by HPLC showed a wide range of loading doses of insecticide wildly off the target dosage. As with PermaNet 2.0, it appears that a high loading dose does not compensate for the stripping off of insecticide or leave sufficient insecticidal efficacy at 20 washes. Less than 2mg ai/m² insecticide was detected after 20 washes, even on nets over doses of insecticide equivalent to more than 300 mg ai/m² in places. Since these tests were carried out the manufacturer of Dawa has ceased production of this long-lasting net candidate.

With LLIN technology now being embraced by control programmes worldwide, there is likely to be a proliferation of manufacturers bringing LLIN products to the market. Several products are currently under development. Draft LLIN specifications have been prepared (FAO/WHO, 2004) but methods for evaluating LLIN need standardization to distinguish LLIN from less durable or even fraudulent LLIN, bearing in mind that most conventional ITNs retain at least some insecticidal power after washing. Gonzales *et al.* (2002) found that soaking and more vigorous washing was needed to differentiate between CTDNs and PermaNet. The work presented here shows that the vigorous water bath wash protocol, adopted *ad interim* for WHOPES purposes (section 3.3.1 in WHO, 2004a), was able to demonstrate a clear difference between the LLIN and the CTDN. For proving the superiority of LLINs over conventionally treated nets in everyday use, or to demonstrate incremental improvements in performance of LLINs, a standardised wash protocol that equates to the most vigorous wash likely to be used in everyday use is required. There is still no consensus on what should constitute a standardised wash for whole nets. Current wash protocols do not incorporate a realistic time interval between washes, whereas in everyday use owners may wash nets infrequently or irregularly at intervals of weeks, months or even years. In accelerated trials, it is convenient to re-wash rapidly, even daily, which may be insufficient interval to allow reactivation of certain types of LLIN such as Olyset® (WHO, 2001a, 2004b) which has insecticide incorporated within the polyethylene

yarn and requires more time to restore potency by diffusion of active ingredient from within the polyethylene to the surface. However, the practicalities of washing LLINs more than 20 times in preparation for testing means that intervals between successive washes should be no more than a week if studies are to be done within a reasonable time frame.

LLIN technology is not just about insecticide wash-resistance but also provision of long-term effectiveness against mosquitoes during regular use. Important points bearing on effectiveness include user acceptability, the physical life-span of the net and treatment, migration of insecticide to the surface and sustained availability during long-term use, interaction with mosquito tarsi and uptake when they stand on the net. Beyond experimental hut and platform trials, another critical LLIN requirement is the retention of sufficient insecticide on nets after several years of use by householders. The PermaNets bioassayed after 20 washes by Gonzales *et al.* (2002) were then given to Colombian villagers for everyday use. When the deltamethrin concentration was assayed after 3 years of domestic usage and washing, with regular washing, the residue had dropped from the original 55 mg/m² to an average of 9.6 mg/m² (Kroeger *et al.* 2004). Even so, bioassay mortality remained at 88%. Unfortunately no CTDNs with similar usage history were available for controlled comparison. Elsewhere, user studies conducted in Malawi and Uganda did compare the earlier PermaNet against CTDN controls, and tests showed marked declines in efficacy and deltamethrin content within 6-24 months of use, less for PermaNet than for CTDN (WHO, 2004a). Results of insectary, overnight tests and chemical assays of PermaNet 2.0 are impressive so far, but the *interim recommendation* conferred by WHOPES (2004a) reflects the need for evidence of long-lasting efficacy over several years of use in different settings.

The results presented here provide adequate evidence that PermaNet™ 2.0 has considerably superior persistence over a period of washing that conventionally treated nets. This net, and the LLIN Olyset®, are extremely useful tools for emergency settings where net retreatment campaigns may well be difficult to carry out and provision of nets to returnees are likely to be lost to any follow-up retreatment services that do exist.

Chapter 9. Efficacy and potential for transmission reduction of chloroquine or sulphadoxine-pyrimethamine alone and in combination with primaquine or artesunate for the treatment of uncomplicated falciparum malaria

9.1 Introduction

Chloroquine resistance (RI) of falciparum malaria has been reported in Pakistan since the 1980s (Khaliq *et al.*, 1985; Fox *et al.*, 1985) and is prevalent in every malarious area examined (Shah *et al.*, 1997; Khan *et al.*, 2004). Despite this, chloroquine remains the national first-line treatment policy (Ministry of Health, Pakistan, 2002). Following a trial documenting high chloroquine resistance in the Afghan refugee camps in the North West of Pakistan, the recommendation was made that sulphadoxine-pyrimethamine (SP), the next most affordable and still relatively efficacious treatment, should be considered as a replacement first-line treatment in the camps (Rowland *et al.*, 1997c). The United Nations High Commissioner for Refugees (UNHCR) is responsible for policy decisions in these camps; whilst it is normal practice for UNHCR to adopt the national treatment policy in refugee settings, when there is good evidence of failing treatment, alternatives may be considered (UNHCR, 1999). Gradual moves from chloroquine to SP were made (HNI, unpublished). However, this may no longer be the most appropriate option; low levels of SP resistance had previously been demonstrated in the region (Rowland *et al.*, 1997c). Current levels of SP resistance are not known and it could be that resistance has risen and spread. Experience with the use of SP in South East Asia has shown that its operational usefulness in areas of low or seasonal endemicity is limited in time due to selection of resistant *pf dhps* and *pf dhfr* alleles as a result of the drug's long plasma half-life (Nosten and Ashley, 2004). In Pakistan and elsewhere in the region transmission is seasonal, so strong immunity seldom develops and almost all infected people become symptomatic and seek treatment. The majority of parasites are therefore exposed to antimalarial drugs, exerting strong selection pressure for the resistance genes (Bloland, 2000).

Artemisinin-based combination therapies (ACT) are a relatively new option. In recent years several trials of artemisinin combinations have been carried out. A recent meta-analysis examined 16 of these and concluded that ACT has the potential to improve

treatment outcomes and inhibit the transmission of malaria in areas where conventional treatment is failing (International Artemisinin Study Group, 2004). This study and its partner study (Durrani *et al.*, 2005) are the first trials to have examined the efficacy of ACT in the sub-continent.

An important factor in a low transmission and epidemic prone setting such as the Afghan refugee camps in Pakistan is the potential impact that certain drug regimens may have on transmission levels. Evidence from the Gambia (Targett *et al.*, 2001) and from Pakistan (Rowland, unpublished) indicates that treatment of clinical infections with SP results in high gametocyte loads over the next 10 days, even though asexual stages are quickly eliminated from the peripheral circulation. The implications of this for transmission make an argument for always combining SP with a second, gametocytocidal anti-malarial in low transmission settings. In Pakistan, the national policy is to co-treat with primaquine when administering chloroquine, to eliminate gametocytes and thereby reduce the chance of transmission. The relevance of this practice, in the light of the increasing CQ resistance, is unclear. Another option to eliminate gametocytes is to use an artemisinin-based combination therapy (ACT). The gametocytocidal activities of artesunate are well documented (von Siedle *et al.*, 2001; Targett *et al.*, 2001; International Artemisinin Study Group, 2004), either as a result of the rapid clearance of trophozoites, or a directly gametocytocidal action (Sutherland *et al.*, 2003). Internationally, co-treatment with primaquine was previously recommended as an option for epidemic response (Najera *et al.*, 1998). Recently use of ACTs for epidemic response or in epidemic prone areas has been recommended (WHO, 2004c). It may be that ACTs are a more effective choice than co-treatment with primaquine in situations where transmission reduction through treatment regimen can play a useful role (low transmission settings) or is an urgent need (epidemic response or in epidemic prone settings such as new refugee or IDP camps). A direct comparison of the gametocytocidal effects of these two drugs has not been made and would be useful.

Changing a treatment policy has considerable health, implementation and cost implications. It is therefore vital that any change is based on reliable data demonstrating the most effective and durable treatment. Efforts to promote a policy change, both nationally and for the refugee camps would benefit from firm evidence for the most appropriate alternative. This study was designed to gather evidence to inform decision making at several levels: guidelines for treatment in the Afghan refugee camps

in Pakistan; Pakistan's national treatment policy; regional recommendations (Iran, Pakistan, Afghanistan), and international epidemic response guidelines.

9.2 Materials and Methods

9.2.1 Study area and population

Patients included in the trial came from five Afghan refugee camps situated within 100km from Peshawar, North-west Frontier Province, Pakistan. These camps have all been established for over 20 years. Houses are made of mud and stone with wooden beams and thatch roofs. Three of the five camps (Adizai, Naguman and Yakka Ghund) are sited on the banks of the river Kabul and largely waterlogged. Crops such as wheat and sugar are grown. Breeding sites include seasonal rivers, irrigation ditches and borrow pits. Two of the camps (M. Khoja and Kotki 1) are situated on dry hillsides where there is little agriculture. The few, isolated breeding sites are mainly small pools. Malaria transmission in the area is seasonal with cases of vivax malaria occurring from March to November and falciparum malaria from July to January.

Each selected camp has a history of falciparum malaria. The camps are all located near to, or are adjoined to, Pakistani villages. Often homologous malaria prevalence between the refugee settlements and local villages suggest a degree of 'parasite exchange' (Rowland *et al.*, 1997c). Local Pakistanis frequently use the camp clinics for health care, although to facilitate follow-up only camp residents were recruited to the study.

Although most malaria in the refugees is thought to be the result of local transmission (Suleman, 1988; Bouma and Rowland, 1994), cross-border movement of Afghan males into and out of Afghanistan is frequent and presumably results in some imported and exported malaria cases. Here the assumption is made that only a small proportion of malaria patients recruited to this study would have acquired their infection in Afghanistan as i) recruitment criteria required four or six weeks of follow-up and would therefore have excluded many of the more frequent travelers and ii) only the adult males are likely to have made cross-border trips (74% of the recruited patients were female or younger than 14 years old).

9.2.2 Study sites

Of the five refugee camps from which patients were drawn there were two pairs of adjacent camps. Three study sites were therefore established, in three of the five camps. Patients from the fourth camp, Naguman, were referred to study site 1 in Adizai and patients from the fifth camp, Kotki 1, were referred to study site 3 in M. Khoja. Study site 2 was established in Yakka Ghund. Study sites were located in camps with functioning, well-used clinics, managed by HealthNet International (HNI) (site 1), The International Rescue Committee (site 2), and the Pakistan government department responsible for refugee health care (site 3). HNI staff were installed at each site to recruit and follow-up the patients, with the routine clinic work carried out by the implementing agency as usual.

The study took place over three malaria seasons: 2000-2001; 2001-2002 and 2002-2003. In the first season only site 1 was used, in the second season site 2 was added and the in the third season site 3 was added. All 6 study arms were run from site 1 in the first season. In the second and third seasons there was a cross-over of treatment arms between sites 1 and 2; in the second season site 1 recruited patients to the three SP arms and site 2 recruited patients to the three CQ arms; this was reversed in the third season. At site 3, introduced in the third season, patients were recruited to the CQ arms only. These details are summarized in Table 9-1.

These choices were driven by the needs of a parallel study (Graham *et al* unpublished), which required processing and storage of biological samples and was therefore dependant on the location of equipment and the local power facilities.

Table 9-1. Summary of treatment arms include in each study site over three transmission seasons of the study.

		Study site		
		Site 1	Site 2	Site 3
Transmission season (July – January)	Season 1: 2000-2001	CQ arms SP arms		
	Season 2: 2001-2002	SP arms	CQ arms	
	Season 3: 2002-2003	CQ arms	SP arms	CQ arms

9.2.3 Study design and procedures

Patients presenting with symptoms of malaria and diagnosed by the routine clinic staff, using microscopy, as falciparum positive were referred to the study staff for further assessment. Consecutive patients were screened for the following inclusion criteria: 1) over 2 years of age; 2) weight over 5kg; 3) not pregnant; 4) *P. falciparum* mono-infection; 5) greater than 1 asexual parasite per 10 fields; 6) no other serious disease (e.g. cardiac, renal or hepatic); 7) understands and is willing to sign the consent form; 8) a camp resident willing to collaborate for a full period of follow-up; 9) reports that no malaria drugs have been taken in the last 21 days; 10) no signs of severe malaria. All patients recruited to the study (or, in the case of children, their parents or guardians) were interviewed by the study supervisor about symptoms, previous anti-malarial therapy and use of other medications. Axillary temperature (measured underarm with an electronic thermometer) and weight were measured. A finger prick was used to take blood for a confirmatory thick and thin film and for a packed cell volume (PCV) test (in sites 1 and 3 only).

Patients meeting the inclusion criteria were assigned to a treatment arm using a pre-prepared pseudo-randomised table sub-divided into sex and age. Patients received either chloroquine ("Nivaquine", Beacon, 150mg tablets); SP ("Fansidar", Roche, 500mg/25mg tablets), chloroquine plus primaquine (7.5 mg tablets); SP plus primaquine; chloroquine plus artesunate (Plasmotrim, Mepha, 50mg tablets), or SP plus artesunate.

Medications were dosed according to modified weight guidelines from WHO for administration of fractions of tablets, all treatments were given orally: chloroquine, 10mg/kg day 0 and 1, 5mg/kg day 2; SP, single dose 25mg/kg sulphadoxine and 1.25mg/kg pyrimethamine on day 0; primaquine single dose 0.5mg/kg on the last day of treatment, i.e. day 2 in the chloroquine plus primaquine regimen and day 0 in the SP plus primaquine regimen; artesunate, 4mg/kg on day 0, 1 and 2.

The study supervisor was not blinded to treatment group. The patients, microscopists and health workers responsible for recording symptoms during follow-up were blinded. Study medicine was distributed and administered at the clinic and all therapy was directly observed. Patients were observed for 30 minutes after administration of medication and the dose was repeated if vomiting occurred.

Information and samples collected on day 0 are described above. Follow-up appointments were scheduled for days 1, 2, 3, 7, 14, 21 and 28. Patients in the three SP arms were scheduled for an extended followed-up on day 35 and day 42 to record late recrudescence which may be frequent following treatment with this drug (Myint *et al.*, 2004). In the final season patients in the CQ arms were also scheduled for 42 days follow-up. Each follow-up consultation consisted of an axillary temperature recording and completion of a standardised symptom history form. Blood was obtained by finger-prick for thick and thin films on each day, PCV on day 28 and for collection of blood spots on filter paper on a weekly basis for PCR analysis. If a patient was considered to have failed on any day a PCV test and filter paper were also collected on that day.

Patients were encouraged to return to the clinic at any time if they felt ill. Patients presenting at the clinic on any day were examined in the same way as on a day of follow-up. Patients who did not return for a scheduled day were visited at home, those who were absent were visited the next day.

Patients were excluded after enrollment for the following reasons: 1) administration of additional anti-malarial drugs; 2) emergence of any concomitant febrile illness that interfered with outcome classification; 3) withdrawal of informed consent; 4) development of severe malaria or danger signs on day 0.

Patients found to be parasitaemic on any day after day 3 were treated with SP (the official second line treatment) or with SP and mefloquine (Fansimef, Roche) for those whose initial treatment was SP based. These patients were referred to a hospital if severe symptoms had developed.

9.2.4 Laboratory tests

PCV was measured with the micro-haematocrit method, because of equipment and power limitations this was only done for patients recruited at sites 1 and 3.

Thick and thin blood smears were stained with 2% geimsa for 30 min. All slides were read by a microscopist who was blind to the treatment groups and clinical outcomes. One microscopist worked at each site. Parasite density was calculated by counting the number of parasites (either trophozoites or gametocytes) per 200 white blood cells (WBC) from the thick blood smear on the assumption of a WBC count of 8000/ μ l. A smear was regarded as negative if no parasites were seen after review of 100 high-powered fields. Thin blood smears were reviewed for non-falciparum infection.

Cross checking of slides from sites 2 and 3 for accuracy of diagnosis, was done by the site 1 microscopist, no errors were seen. A comparison of parasite counts of the same slides by the three microscopists was done. Less than 5% variation in counts was seen between the 3 microscopists, with no consistent differences.

9.2.5 Outcome measures

The primary endpoints of the study were clinical and parasitological outcomes on day 28. Although a subset of patients were followed-up to day 42 there were few additional failures in the extended follow-up; for ease of comparison across arms and to maximize use of data from patients who were excluded in the additional 2 weeks, day 28 outcomes have been presented throughout (with details of outcomes following 42 day follow-up reported in the text). Patient outcomes were assessed using the WHO classifications systems for treatment outcomes (WHO, 2002), described here as “clinical outcomes”. These are classified as success (adequate clinical and parasitological response), early treatment failure or late treatment failure (which may be late clinical failure or late parasitological failure). Purely parasitological responses are defined using the WHO parasitological outcomes classification system of sensitive (S) or resistant (RI, RII, RIII) infections.

The definitions of clinical and parasitological failure meant that, depending on both the day of failure and the presence or absence of fever, some patients meeting the criteria for parasitological failure were not then evaluable for clinical outcomes and *vice versa*.

Other characteristics of treatment response examined included resolution of fever (with fever defined as axillary temperature $\geq 37.5^{\circ}\text{C}$), trophozoites clearance, gametocyte clearance and gametocyte carriage on or after day 7 after treatment.

9.2.6 Statistical analysis and sample size calculations

Statistical analysis

Treatment arm comparisons of interest were determined *a priori*. Each monotherapy was compared to the related artesunate combination for clinical and parasitological outcomes (after 28 days follow-up). For presence of gametocytes on or after day 7 after treatment, each monotherapy was compared with both the related primaquine combination and the related artesunate combination; in addition the primaquine combination was compared to the related artesunate combination.

Proportions are reported with 95% confidence intervals and treatment comparisons of interest are compared using Chi squared analysis. Odds ratios of treatment failure on or by specified days after treatment were calculated using logistic regression adjusted for age and day 0 trophozoite density (sex was demonstrated by ANOVA to have no affect on treatment failure).

Risk of clinical or parasitological failure, censored at weekly intervals during follow-up, were estimated with Kaplan-Meier survival analysis techniques.

All data were entered in Microsoft Excel (1997), the relatively small amount of data allowed for verification by checking 100% of the data once entered. Analyses were performed using STATA statistical software version 6.0. A *P* value of 0.05 or less was judged significant with qualifications where appropriate.

Sample size calculations

Sample sizes were calculated to demonstrate a difference between pairs of combinations only (each monotherapy compared with either the PQ or AS combination).

Assumptions for sample size (confidence level 95%, power 80%) in the chloroquine combinations were: i. estimated frequency of recrudescence in the chloroquine group: 30%; expected recrudescence in each combination group (CQ+PQ or CQ+AS): 10% or less; ii). estimated prevalence of gametocyte positives after 7 days in the chloroquine group: 50%; in each combination group: 25%.

Assumptions for sample size (confidence level 95%, power 80%) in the SP combinations were: i. estimated frequency of recrudescence in the SP group: 10%, expected recrudescence in each combination group (SP+PQ or SP+AS): 1%; ii) estimated prevalence of gametocyte positives after 7 days in the SP group: 50%; in each combination group: 25%.

For the outcome of recrudescence required samples sizes were 64 in the CQ arms and 121 in the SP arms. For gametocyte carriage required sample sizes were 65 in all arms. Targets for recruitment were therefore 65 in the CQ arms and 121 in the SP arms.

9.2.7 Ethics

The protocol of the study was approved by the London School of Hygiene and Tropical Medicine ethical committee. Written approval to perform the trial was obtained from the Pakistan Medical Research Council.

9.3 Results

9.3.1 Recruitment and follow-up

Numbers of malaria cases were lower during the trial than in previous years. In the CQ arms the actual number of patients completing follow-up in each arm was close to the target sample size. However, in the SP arms the sample sizes were much lower than those which would have had 80% power to demonstrate the size of differences in recrudescence and gametocyte carriage estimated by the sample size calculations and would have had only 25% and 50% power, respectively, to demonstrate the differences in recrudescence and gametocyte carriage specified in the sample size calculations. The magnitude of differences in both failure rates and in gametocyte persistence were, however, larger than estimated and statistical significance was demonstrable.

355 cases of microscopically confirmed falciparum malaria were enrolled into the study. 308 patients (87%) were evaluable for clinical outcomes and 292 for parasitological outcomes after 28 days of follow-up. Some patients were not evaluable for both depending on the day of failure and clinical state at that time. Of the 266 patients recruited into an extended period of follow-up 221 (83%) were evaluable for clinical and parasitological outcomes after 42 days of follow-up (Figure 9-1).

In the subset of patients enrolled into the extended follow-up period of 42 days recrudescence in the additional 2 weeks was low. Only 8 patients were classified as having an adequate clinical and parasitological response (ACPR) or a sensitive infection (S) at day 28, subsequently failed in the extended 2 weeks follow-up (1 in the CQ arm, 2 in the CQ+PQ arm, 3 in the CQ+AS arm, 1 in the SP arm and 1 in the SP+PQ arm); 42 day failure rates were therefore similar to 28 day failure rates for all treatment groups. As numbers recruited were fairly low and there was some drop-out in the 28 - 42 day period, outcomes for all treatment arms at 28 days of follow-up have been presented throughout this chapter.

Characteristics of patients recruited into the 3 CQ treatment arms (Table 9-2) were similar, although those enrolled into the CQ+AS group were less likely to be carrying gametocytes. Characteristics within the three SP arms were broadly similar on admission. Those enrolled in the SP arm were more likely to have gametocytes and to have to have higher gametocyte densities. Patients enrolled in the SP+AS arm tended to be carrying higher numbers of trophozoites. Patients enrolled into the SP arms tended to be older, and therefore heavier, with less likelihood of fever than those enrolled into the CQ arms. Patients were recruited at three different study sites, study site was shown to have no effect on failure rate in either the chloroquine group of treatment arms ($\text{Chi}^2=2.04$, $P=0.361$) or the SP group of treatment arms ($\text{Chi}^2=1.42$, $P=0.234$).

9.3.2 Response to treatment

Clinical and parasitological outcomes are shown in Table 9-3. Fever tends not to be associated with parasite recrudescence during this study; the majority of patients classified as "late treatment failure", under the clinical definitions, fell into the category of "late parasitological failure" in the absence of documented fever. Within each arm, there was little difference in failure rates by clinical outcomes and those classified as failures (RI-RIII) by the purely parasitological definitions.

Resistance to chloroquine is high; fewer than 25% of patients were cured by chloroquine monotherapy. Use of this drug in combination with artesunate gives a cure rate of 72%, a significant improvement over the monotherapy ($\text{Chi}^2=37.60$, $P<0.001$), patients treated with the monotherapy have significantly higher odds of failing at each weekly point of follow-up (Table 9-4).

Treatment failure occurred in a small number of patients following treatment with SP monotherapy, in the respective artesunate combination arm all patients responded adequately to the treatment and only one patient (2%) presented with parasitological failure (RII). However, with SP alone achieving a high cure rate it is not possible to demonstrate a significant difference between the monotherapy and the combination in terms of overall cure rate ($\text{Chi}^2=1.92$, $P=0.166$), or odds of failing at each weekly point after treatment (Table 9-4).

Treatment outcomes for primaquine combinations versus monotherapies were not an important study endpoint and were not examined statistically.

Addition of artesunate to the monotherapies resulted in an improved clinical response with faster clearance of asexual parasites (Figure 9-2) and resolution of fever in more patients over the first 3 days, this difference was most notable in the SP arms (Figure 9-3).

Treatment arm is a significant factor affecting presence of trophozoites at each of these follow-up points ($\text{Chi}^2 > 30.0$, $P < 0.001$ at each follow-up point). Treatment arm has a significant effect on whether a patient is feverish by the second day after treatment (day 2 $\text{Chi}^2 = 15.30$, $P = 0.009$; day 3 $\text{Chi}^2 = 16.5$, $P = 0.006$). Patients receiving SP monotherapy or SP+AS are least likely to have fever by day 3.

The cumulative incidence of therapeutic failure was calculated using survival analysis (WHO, 2003b). The Kaplan Meier survival curves (Figure 9-4) highlight the large difference in outcomes between the seriously failing chloroquine and the still fairly efficacious SP. Figure 9-4 illustrates that the addition of AS improved the outcome when combined with CQ but that over the 28 day period cumulative incidence of failure was still unacceptable.

9.3.3 *Potential for transmission reduction*

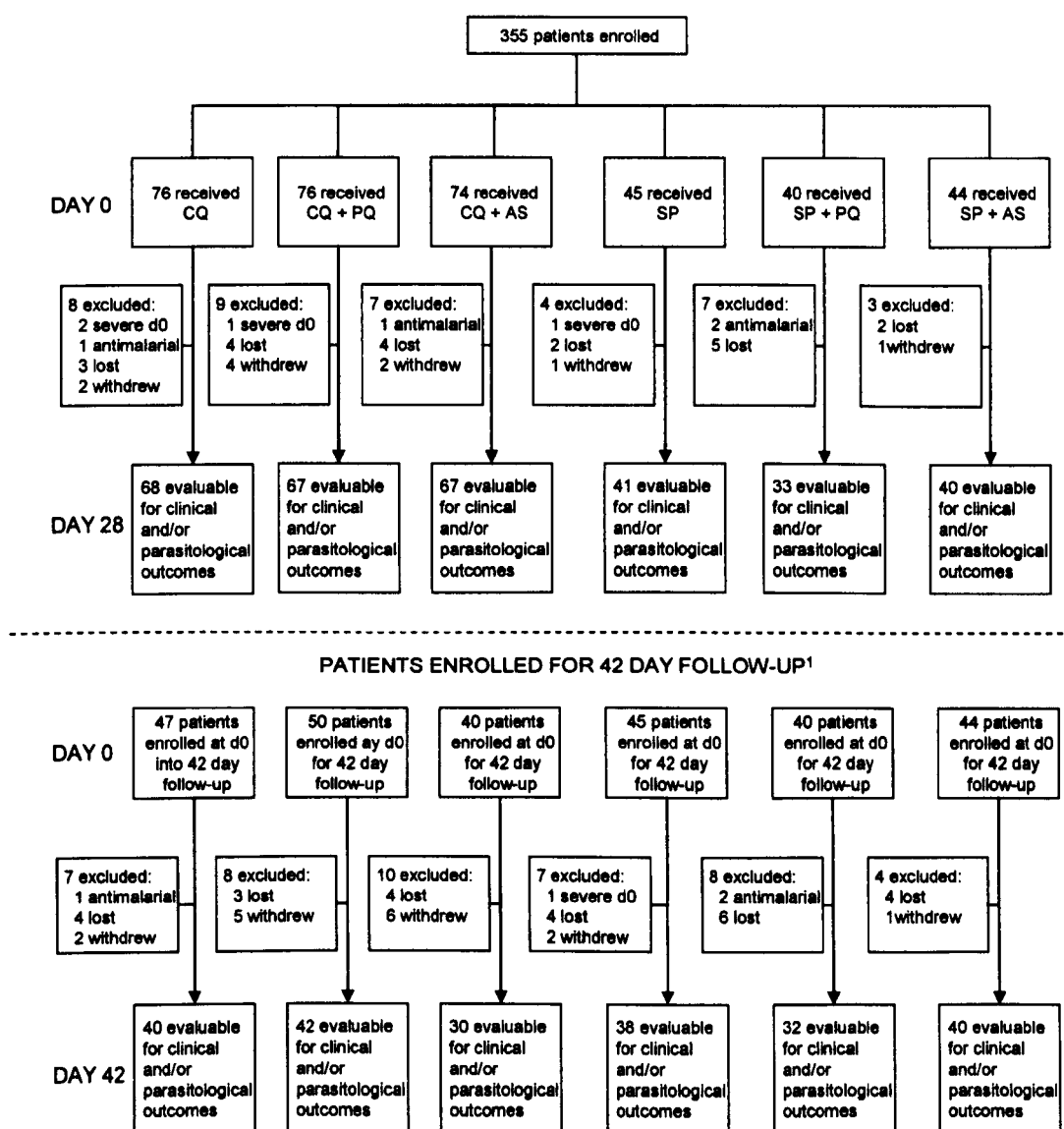
Interpretation of Figure 9-5 must take into account that the percentage of patients with gametocytes on day 0 was higher for the SP monotherapy arm than for the other arms. Nevertheless, it is apparent that the percentage of patients carrying gametocytes in the SP arm rises more rapidly over the first 3 days and shows a slower decline over the day 7 to 28 period, than for any of the other drug regimens. The percentage of patients carrying gametocytes following treatment with chloroquine follows a similar pattern to that in the SP arm, presumably because of the high numbers of patients for whom the chloroquine treatment failed.

An inspection of Figure 9-5 suggests that primaquine does reduce the proportion of patients carrying gametocytes when given as co-treatment with either monotherapy. However, the effect appears to be more dramatic for chloroquine than for SP, and to be delayed until after day 3 for chloroquine and after day 7 for SP. The percentage of patients carrying gametocytes is lowest in the two arms in which patients were treated with artesunate, and this difference is seen after just 2 days of starting treatment. For the SP arms in particular, the addition of AS to the treatment regimen has a clear effect, reducing the proportion of patients carrying gametocytes by a considerable margin.

The odds ratios for carrying gametocytes on or after day 7 in two comparison arms support these observations (Table 9-5). The odds of a patient having persisting gametocytes is higher in either of the monotherapy arms than in both the respective primaquine or artesunate combination arms. The odds ratio is higher when the monotherapies are compared to the artesunate combination rather than the primaquine combination, suggesting that co-treatment with artesunate succeeds in eliminating gametocytes more effectively, in comparison to the monotherapy, than co-treatment with primaquine.

Having gametocytes on day 0 increases the likelihood of patients having gametocytes on or after day 7, regardless of treatment ($\text{Chi}^2=21.2$, $P<0.001$). The most dramatic differences in gametocyte carriage, are seen between patients treated with the artesunate combination compared to the monotherapy when the patients were not carrying gametocytes at the time of treatment (Table 9-6).

Peak gametocyte densities are seen to occur 7 days after treatment with either chloroquine or SP monotherapy (Figure 9-6, A(i) and B(i)). The mean density is more than three times greater following SP treatment than CQ treatment. Both primaquine and artesunate reduce the mean density to similarly low levels. A closer look at the pattern of gametocyte densities (Figure 9-6, A(ii) and B(ii)) shows that following administration of SP, artesunate is effective at maintaining low levels of gametocytes, whereas the day 7 peak, although at on a reduced scale, is still apparent following co-treatment with primaquine.

**Figure 9-1. Trial profile.**

Note:

1. Patients in the three SP arms were scheduled for an extended followed-up on day 35 and day 42 to record late recrudescence which may be frequent following treatment with this drug, in the final season patients in the CQ arms were also scheduled for 42 days follow-up.

Table 9-2. Baseline Characteristics

Variable	CQ	CQ + PQ	CQ + AS	SP	SP + PQ	SP + AS	Total
Number enrolled	76	76	74	45	40	44	355
Number evaluable for parasitological outcomes ¹	63	65	67	40	32	41	308
Number evaluable for clinical outcomes ¹	56	58	67	41	30	40	292
Age [median (IQR) years]	12 (8 - 17.5)	12 (8 - 20)	12 (8 - 20)	17 (9 - 27)	14 (7 - 25)	18.5 (9.5 - 30)	13 (8 - 25)
Percentage female	42	37	50	33	43	45	42
Weight [median (IQR) kg]	28.5 (20 - 47)	30 (19.5 - 52.5)	33 (20 - 45)	48 (25 - 58)	42 (21.5 - 55)	41 (21 - 57)	35 (20 - 52)
Temperature [mean (SD) °C]	37.3 (1.0)	37.5 (1.2)	37.4 (1.2)	37.5 (1.0)	37.5 (1.2)	37.5 (1.5)	37.4 (1.2)
Temperature ≥ 37.5°C on presentation [n (%)]	33 (43)	34 (45)	34 (46)	21 (47)	17 (43)	23 (52)	162 (46)
PCV [mean (SD) % haematocrit] ²	42.9 (9.7)	40.8 (3.9)	41.5 (4.3)	44.2 (7.7)	45.5 (6.9)	44.3 (5.1)	43.2 (6.4)
PCV < 30% [n (%)] ²	0	0	0	1 (2.2)	0	0	1 (0.002)
Asexual parasite density [geometric mean (95% CI) per µl]	5161 (3536 - 7535)	5263 (3647 - 7595)	7366 (4972 - 10,915)	7600 (5185 - 11,140)	8091 (4236 - 15,454)	12,134 (7757 - 18,982)	6856 (5773 - 8143)
Gametocyte positive [n(%)]	12 (15.8)	15 (19.7)	9 (12.2)	14 (31.1)	7 (17.5)	7 (15.9)	64 (18.0)
Gametocyte density [geometric mean (95% CI) per µl]	1.3 (0.4 - 2.6)	1.7 (0.7 - 3.4)	0.6 (0.2 - 1.2)	4.1 (1.4 - 10.1)	1.6 (0.3 - 4.1)	1.2 (0.2 - 2.9)	1.5 (1.0 - 2.0)

Notes: (1) The total number of patients evaluable for either clinical or parasitological outcomes is not the same: patients failing clinically on days 1, 2 or 3 are not then evaluable parasitologically, as patients must be evaluable up to day 7 for parasitological outcomes. Patients failing parasitologically on day 7, but not failing clinically by that day are not evaluable for clinical outcomes. (2) PCV was not recorded for all patients; a microcentrifuge was only available at one of the 3 clinics. For PCV percentages in the 6 treatment groups: CQ n=10; CQ+PQ n=19; CQ+AS n=13; SP n=19; SP+PQ n=15, SP+AS n=15.

Table 9-3. Clinical and parasitological outcomes after 28 days follow-up: n(%)

	CQ	CQ + PQ	CQ + AS	SP	SP + PQ	SP + AS
Clinical outcomes	N = 56	n = 58	n = 67	n = 41	n = 30	n = 40
Adequate clinical response	13 (23%)	18 (31%)	48 (72%)	37 (90%)	28 (93%)	40 (100%)
Early treatment failure	11 (20%)	5 (9%)	0	1 (2%)	1 (3%)	0
Late clinical failure	32 (57%)	35 (60%)	19 (28%)	3 (7%)	1 (3%)	0
Parasitological outcomes	N = 63	n = 65	n = 67	n = 40	n = 32	n = 41
S	13 (21%)	18 (28%)	48 (72%)	38 (93%)	28 (88%)	40 (98%)
RI	36 (57%)	35 (54%)	19 (28%)	3 (7%)	1 (3%)	0
RII	12 (19%)	9 (14%)	0	0	3 (9%)	1 (2%)
RIII	2 (3%)	3 (5%)	0	0	0	0

Note:

The total number of patients evaluable for either clinical or parasitological outcomes varies: patients failing clinically on days 1, 2 or 3 are not evaluable parasitologically as patients must be evaluable up to day 7 for parasitological outcomes. Patients failing parasitologically on day 7, but not failing clinically by that day are not evaluable for clinical outcomes.

Table 9-4. Adjusted odds ratios for treatment failure on or by specified intervals after treatment with monotherapy and the equivalent artesunate combination

Treatment comparison	Day 7		Day 14		Day 21		Day 28	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
CQ vs. CQ + AS	34.9 (4.4 - 277.2)	0.001	20.1 (6.9 - 58.7)	< 0.001	15.9 (6.3 - 39.7)	< 0.001	16.2 (6.4 - 41.1)	< 0.001
SP vs. SP + AS	13.4 (0.1 - 2245.1)	0.32	13.4 (0.1 - 2245.1)	0.32	9.8 (0.3 - 319.5)	0.198	19.0 (0.4 - 825.0)	0.126

Notes:

1. Odds ratios are adjusted for age, sex and trophozoite density on day 0.
2. Treatment failure is defined as either parasitological or clinical failure, whichever occurs sooner.

Table 9-5. Adjusted odds ratios for gametocyte carriage on or after day 7 after treatment with study drugs.

Treatment comparison	OR (95% CI)	<i>P</i>
CQ vs. CQ + PQ	8.9 (3.8 - 20.4)	<0.001
CQ vs. CQ + AS	33.9 (12.2 - 94.1)	<0.001
CQ + PQ vs. CQ + AS	2.6 (1.1 - 5.7)	0.022
SP vs. SP + PQ	3.1 (0.8 - 11.3)	0.092
SP vs. SP + AS	45.4 (10.7 - 191.7)	<0.001
SP + PQ vs. SP + AS	14.6 (4.3 - 49.0)	<0.001

Note:

Odds Ratios are adjusted for age and sex

Table 9-6. Percentages of patients with gametocytes on or after day 7 after treatment, in those with and without patent gametocytaemia on day 0.

	Patients with patent gametocytaemia on day 0		Patients without patent gametocytaemia on day 0	
	No. of patients with gametocytes on or after day 7, of those with patent gametocytaemia on day 0 (%)	χ^2 of the combination regimen (+PQ or +AS) compared to the complementary monotherapy	Number of patients with gametocytes on or after day 7, of those without patent gametocytaemia on day 0 (%)	χ^2 of the combination regimen (+PQ or +AS) compared to the complementary monotherapy
CQ	11 / 12 (91.7%)		47 / 56 (83.9%)	
CQ + PQ	7 / 14 (50%)	$\chi^2 = 5.3$; $P = 0.022$	21 / 56 (37.5%)	$\chi^2 = 25.3$; $P < 0.001$
CQ + AS	8 / 9 (88.9%)	$\chi^2 = 0.1$; $P = 0.830$	7 / 63 (11.1%)	$\chi^2 = 63.4$; $P < 0.001$
SP	14 / 14 (100%)		25 / 29 (86.2%)	
SP + PQ	5 / 7 (71.4%)	$\chi^2 = 4.4$; $P = 0.035$	23 / 30 (76.7%)	$\chi^2 = 0.9$; $P = 0.347$
SP + AS	5 / 7 (71.4%)	$\chi^2 = 4.4$; $P = 0.035$	5 / 37 (13.5%)	$\chi^2 = 34.7$; $P < 0.001$

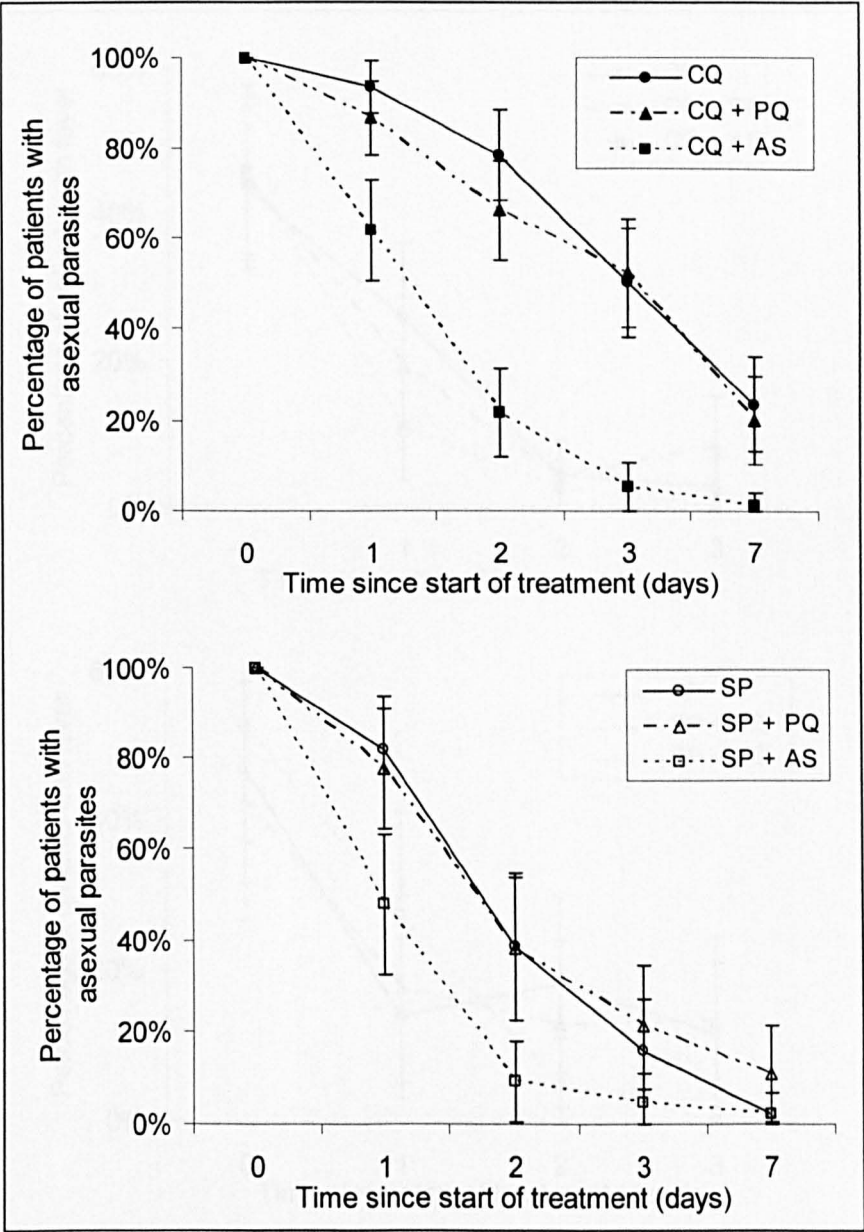


Figure 9-2. Percentage of patients carrying asexual parasites at specified days after treatment.

Note: Error bars show the 95% confidence intervals.

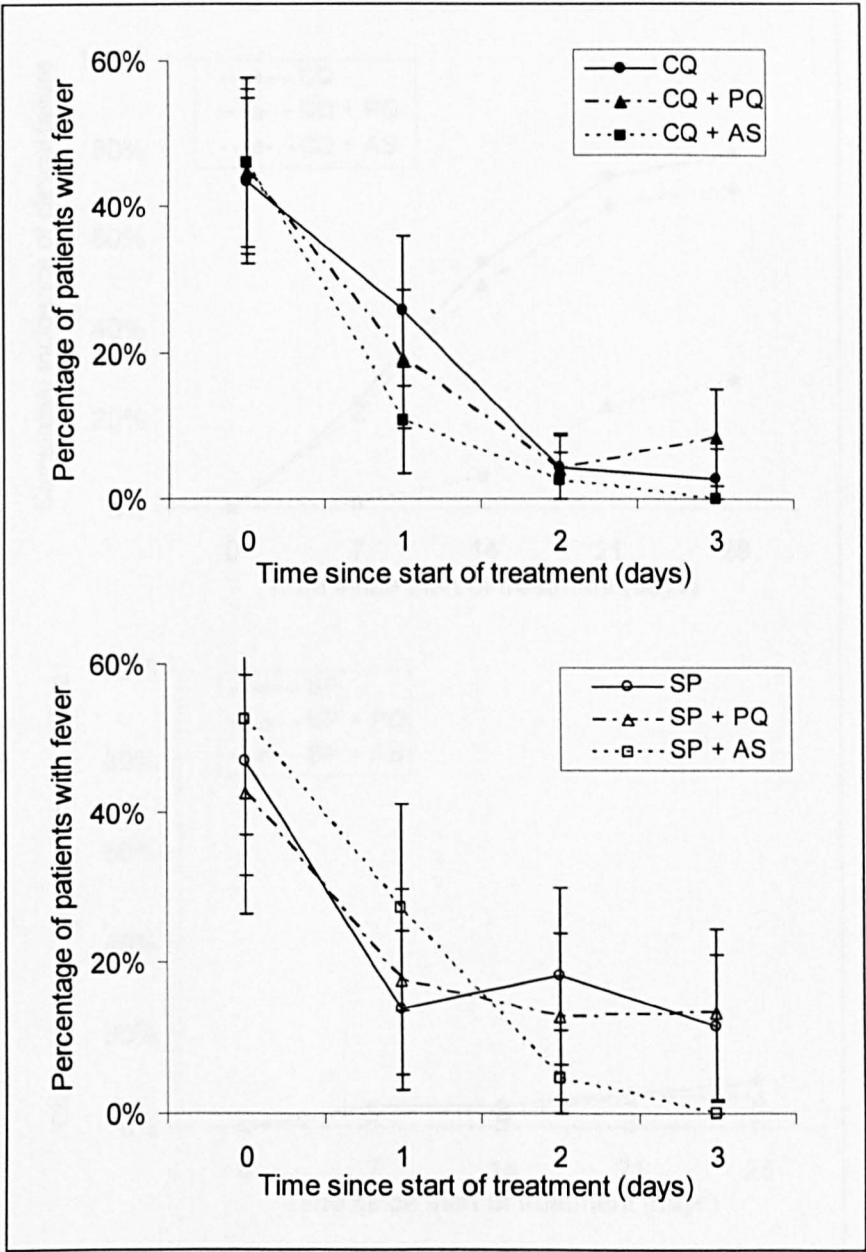


Figure 9-3. Percentage of patients with fever at specified days after treatment.

- Notes:
1. Fever is defined as axillary temperature $\geq 37.5^{\circ}\text{C}$
 2. Error bars show the 95% confidence intervals.

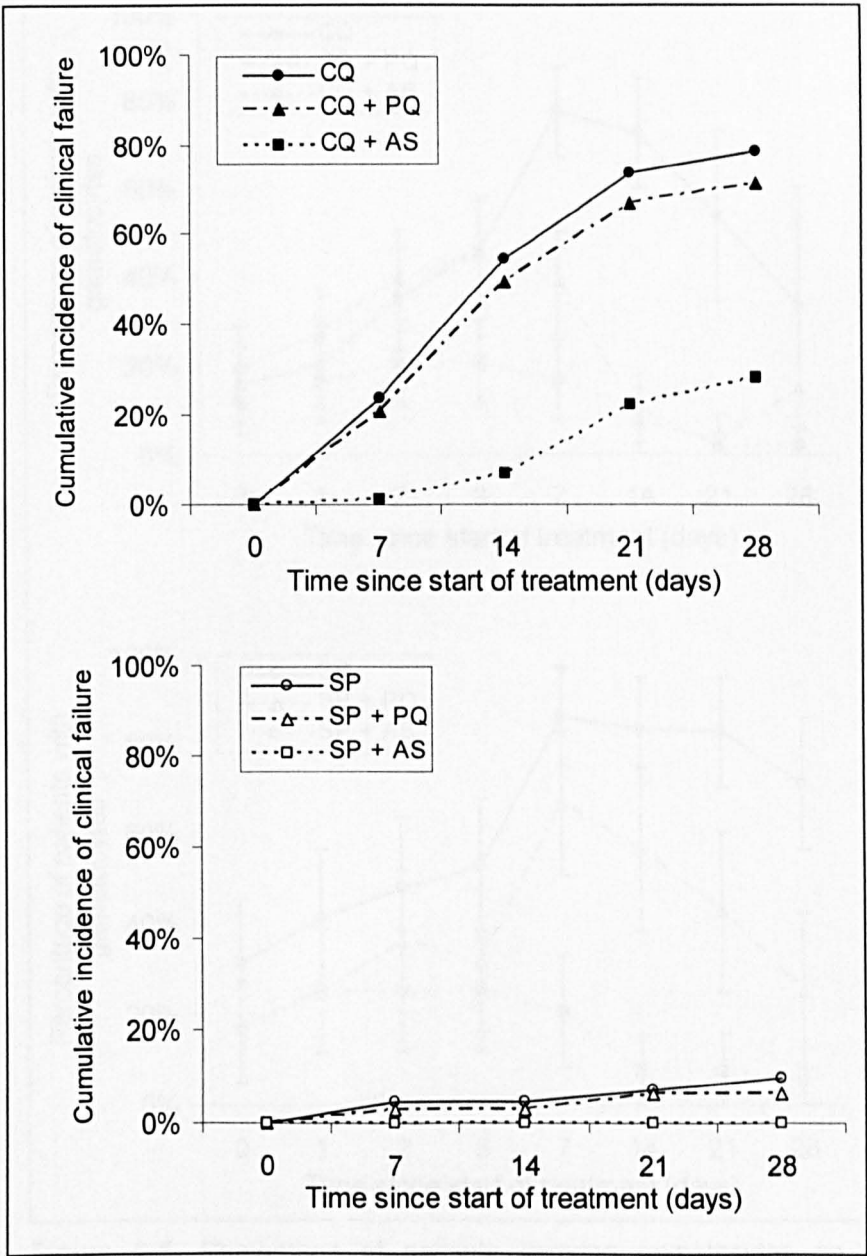


Figure 9-4. Cumulative incidence of clinical failure.

Note: Data were censored at weekly intervals, *i.e.* all those who failed in day 1-7 are classified as incident failures at day 7.

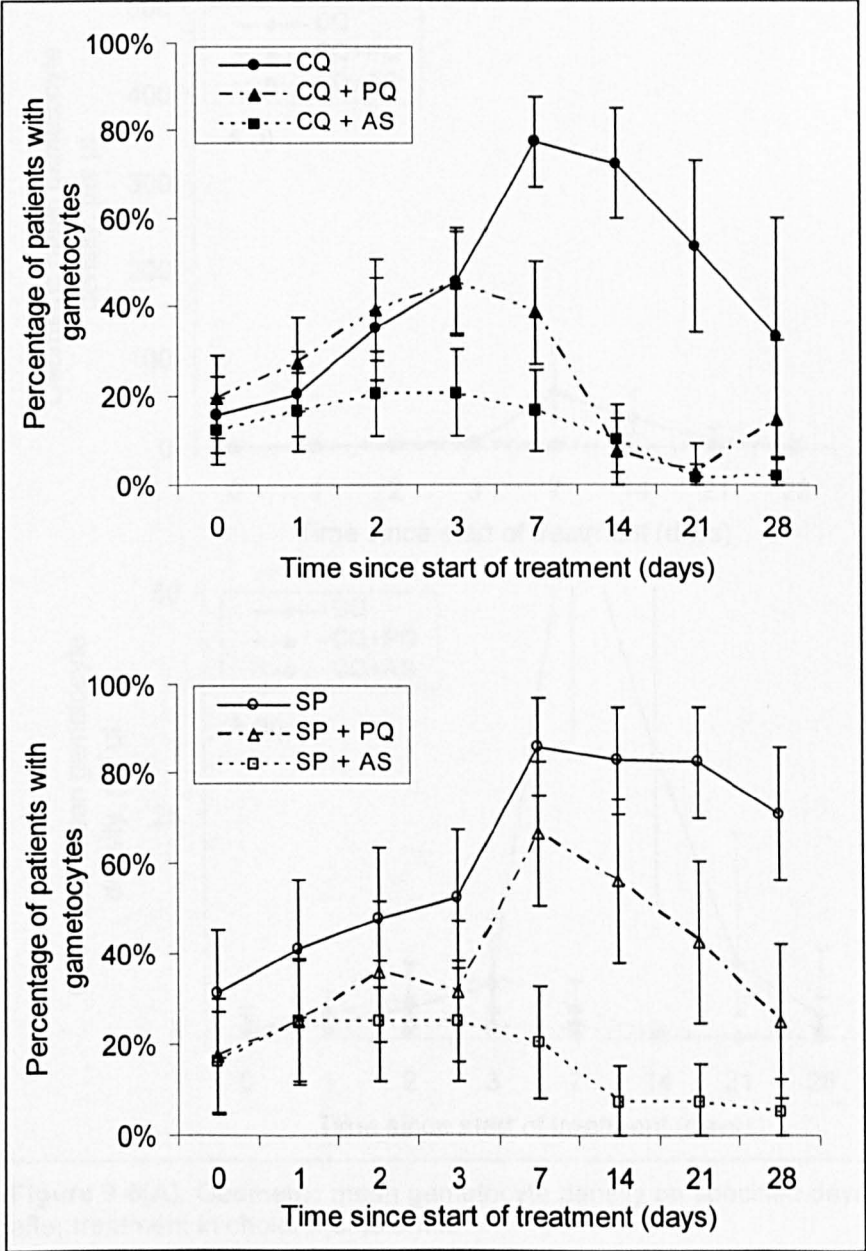


Figure 9-5. Percentage of patients carrying gametocytes on specified days after treatment

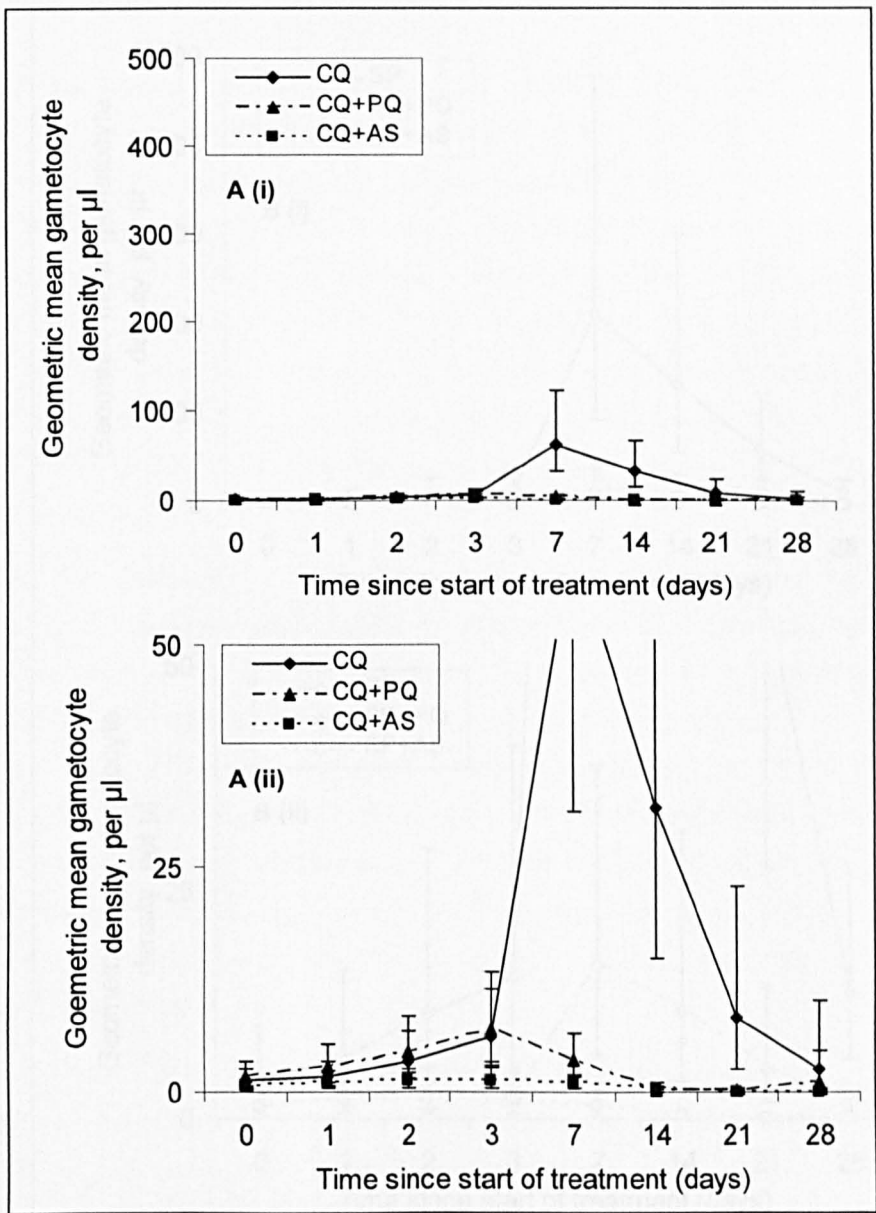


Figure 9-6(A). Geometric mean gametocyte density on specified days after treatment in chloroquine arms.

(SP arms are shown in Figure 9-6(B) overleaf)

Figure (i) is drawn with the y-axis scale to 500 gametocytes per µl; Figure (ii) is drawn with the y-axis scale to 50 gametocytes per µl, to allow inspection of the changes at lower densities.

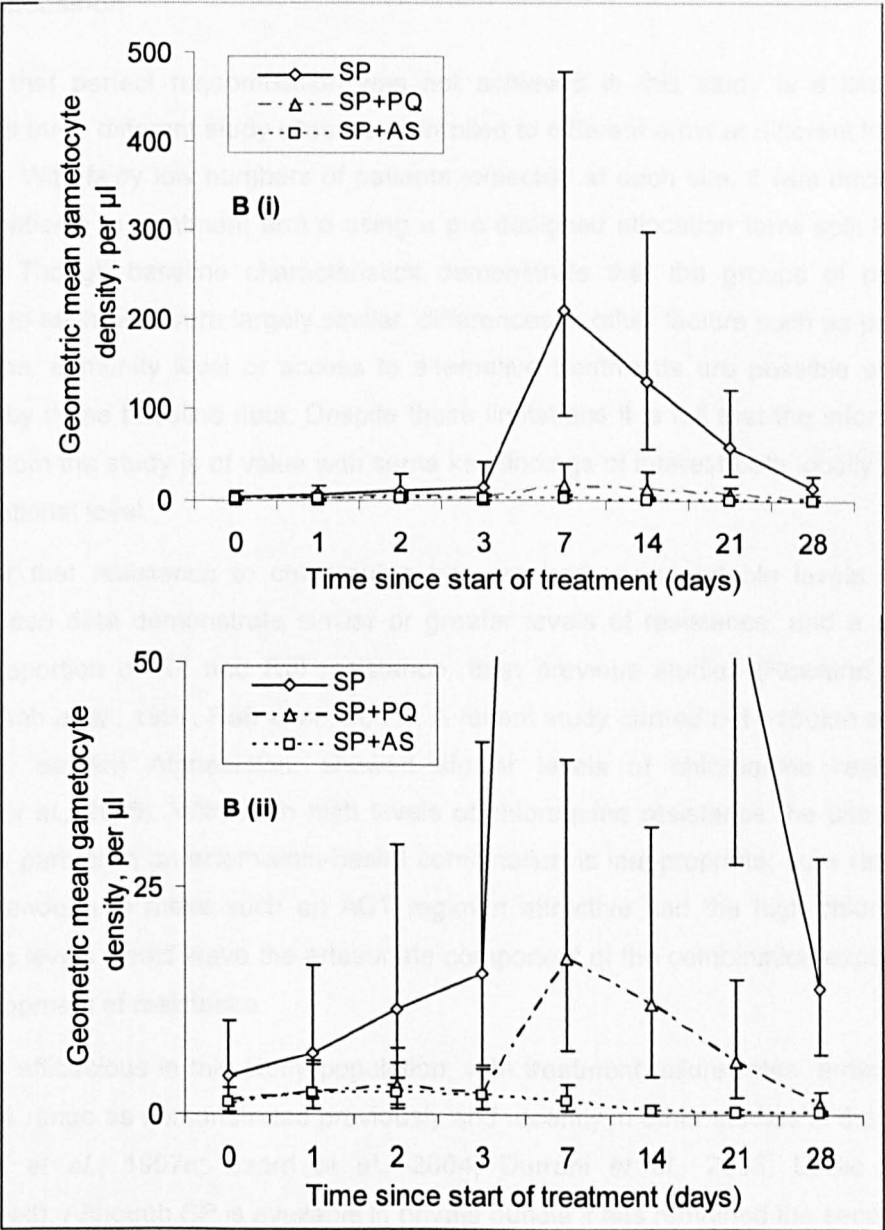


Figure 9-6(B). Geometric mean gametocyte density on specified days after treatment in SP arms.

(CQ arms are shown in Figure 9-6(A) on the previous page)

Figure (i) is drawn with the y-axis scale to 500 gametocytes per µl; Figure (ii) is drawn with the y-axis scale to 50 gametocytes per µl, to allow inspection of the changes at lower densities.

9.4 Discussion

The fact that perfect randomisation was not achieved in this study is a limitation. Patients at three different study sites were enrolled to different arms at different times of the study. With fairly low numbers of patients expected at each site, it was decided to allocate patients to treatment arm a using a pre-designed allocation table split by age and sex. Though baseline characteristics demonstrate that the groups of patients allocated to each arm were largely similar, differences in other factors such as parasite populations, immunity level or access to alternative treatments are possible and not revealed by these baseline data. Despite these limitations it is felt that the information gleaned from the study is of value with some key findings of interest both locally and at an international level.

It is clear that resistance to chloroquine has reached unsupportable levels in this region; these data demonstrate similar or greater levels of resistance, and a slightly higher proportion of RII and RIII resistance, than previous studies (Rowland *et al.*, 1997c; Shah *et al.*, 1997; Rab *et al.*, 2001). A recent study carried out ~150km away in Jalalabad, eastern Afghanistan, showed similar levels of chloroquine resistance (Durrani *et al.*, 2005). With such high levels of chloroquine resistance the use of this drug as a partner in an artemisinin-based combination is inappropriate; cure rates are not high enough to make such an ACT regimen attractive and the high chloroquine resistance levels would leave the artesunate component of the combination exposed to the development of resistance.

SP is still efficacious in this study population, with treatment failure rates remaining in the 5-10% range as demonstrated previously and recently in other studies in the region (Rowland *et al.*, 1997c; Ezard *et al.*, 2004; Durrani *et al.*, 2005; Leslie *et al.*, unpublished). Although SP is available in private outlets it has remained the second line treatment for falciparum malaria, and therefore has not been used to the same extent as a first-line national treatment. The SE Asian experiences of the rapid rise in SP resistance in settings of similar endemicity (Nosten and Ashley, 2004), leads to the concern that there may be a rapid development of resistance to SP if this were chosen as the national first line treatment, which would result in the need for a further policy change in the near future.

The efficacy and potential for resistance is not the only consideration when choosing a treatment policy. In areas of low or moderate transmission it is feasible to use some drug regimens as a tool for transmission reduction. This study setting is characterized by low endemicity and semi-immunity, the majority of malaria infections therefore become symptomatic cases. In such a setting most infectious mosquito bites will lead to a patient seeking treatment. *Plasmodium falciparum* gametocytes usually appear in the blood stream after development of symptoms and, therefore, usually after treatment. In order for a treatment policy to be reasonably expected to have an impact on transmission there are several criteria which would need to be met: i) low to moderate transmission (leading to semi-immunity meaning that most infectious mosquito bites lead to symptoms and treatment seeking); ii) the majority of the population use public health facilities rather than private facilities in which there will be no, or little, control of the prescribed drug regimens; iii) the public health facilities are correctly prescribing the assigned drug regimen, and iv) patients are taking the full course of the prescribed drug regimen.

In this chronic emergency setting, reasonably good health care facilities are available in every camp. Whilst these are thought to be well utilized by the Afghan refugees and the nearby local Pakistani population, use of private clinics is increasingly common in some areas and the true proportion of cases seeking treatment in public health facilities is not known. In many acute emergency, camp environments the majority of the population can be reasonably expected to make use of the free on-site healthcare which is usually provided. Where security outside the camps is poor, this can be assumed with more assurance. In such settings the advantage of a treatment regimen which lowers local transmission levels could be realized, and could be a valuable intervention in what is often an epidemic prone environment. The degree of parasite exchange between the camp and local populations (which may receive a different drug regimen in public health facilities and are more likely to be accessing private health facilities) will of course have an impact. On a national level the reduction of transmission is only likely to be a sensible objective of a national treatment policy if the country has defined areas of transmission where the majority of the population resorts to public healthcare rather than to private clinics, in which there is unlikely to be any control over the type of drug administered.

No models have been published defining the limits, in settings of varying transmission, of gametocyte density and days of gametocyte carriage which would have an impact on transmission; such work would be useful. However, documenting gametocyte carriage rates and densities is the first step in predicting the *comparative* potential of treatments to lower transmission; gametocyte density has been shown to be strongly correlated with transmission success (Drakely *et al.*, 1999) and the length of time that the patient has gametocytes in their blood increases the likelihood that these sexual stages are picked up by a feeding mosquito.

Treatment with either the clinically effective SP or the failing chloroquine resulted in persistence of gametocytaemia into or after the second week after administration of treatment, in over 80% of cases. As expected, this was higher for SP than for CQ; SP is known to result in high rates of gametocyte carriage after treatment (von Seidlein *et al.*, 2001). However, as observed in a recent trial in East Afghanistan (Durrani *et al.*, 2005), this difference was slight. This contrasts with situations where the frequency of 4-aminoquinoline resistance is lower and prevalence of gametocytaemia following treatment tends to be lower (Robert *et al.*, 2000; von Seidlein *et al.*, 2001). These findings confirm that SP is simply not effective as gametocytocidal agent, and suggest that the effect of 4-aminoquinolines on young gametocytes is lost when the parasites become resistant (Robert *et al.*, 2000).

The gametocytocidal action of artemisinin derivatives are well documented (von Seidlein *et al.* 2001; International Artemisinin Study Group, 2004) and artesunate proved highly effective in reducing the prevalence of gametocytaemia in this study. Regimens combining artesunate with CQ or SP saw dramatic reductions in gametocyte carriage; fewer than 25% of cases had persisting gametocytaemia in these artesunate combination arms. As von Seidlein and colleagues found for a SP+AS regimen in The Gambia (2001), the drugs under investigation here also appear to have limited activity against mature circulating gametocytes, which tend to be relatively metabolically inactive (Butcher, 1997). Both primaquine and, more dramatically, artesunate reduce the odds of persisting gametocytes from that seen following treatment with either CQ or SP, they appear to do so by acting against the younger gametocytes which are probably sequestered at the time of presentation and therefore not detected (von Seidlein *et al.*, 2001).

These results hold with the findings of the recent International Artemisinin Study Group meta-analysis that “the addition of 3 days of artesunate to standard anti-malarial treatments substantially reduces treatment failure, recrudescence, and gametocyte carriage” (International Artemisinin Study Group, 2004). The present study showed that the addition of artesunate to a seriously failing adjuvant drug does not reduce treatment failure to acceptable levels. Here the addition of artesunate to the failing chloroquine increased the cure rate from ~25% to 72%, a substantial increase but still an unacceptable level of failure and a regimen which falls within the “change period” range for policy change (WHO, 2001b). This finding is in contrast to a recent study where the addition of artesunate to the failing amodiaquine increased the cure rate from 28% to 92% (Durrani *et al.*, 2005).

Introduction of an artemisinin-based combination as the first line treatment for confirmed falciparum malaria in this region would have the benefit of ensuring the most effective drug regimen is prescribed and may have the additional benefits of reducing transmission in some areas and preventing the development of resistance to another drug and therefore the need for a further drug policy change. Following the presentation of the results of this study and a concurrent study in East Afghanistan (Durrani *et al.*, 2005) the WHO Eastern Regional Office recommended SP+AS as the first line treatment in this region (Pakistan, Afghanistan and Iran) (WHO-EM, 2004). However, the current shortage of artemisinin-based drugs may be one factor that could undermine this policy change.

There has been extremely emotive and influential advocacy for ACT drug regimens to be put in place in all countries where resistance to the current first line has been proven (*e.g.* MSF, 2003; Attaran *et al.*, 2004). Most governments are now able to access the necessary funds to support the introduction of ACT, under the Global Fund for AIDS Tuberculosis and Malaria (GFATM). As a result, a massive increase in orders (and predicted orders) for artemisinin derivative drugs has led to a worldwide shortage of the raw material, Artemisia. In light of this situation hard decisions need to be made about the use of the available drugs. Countries with a heavy malaria burden and a multi-drug-resistant parasite population should be the first in line to receive ACT. Although there is no question that chloroquine must be replaced as a first-line treatment for malaria in this region, the country may have to consider moving to a first line treatment policy of SP as an interim solution if artesunate is unavailable.

Chapter 10. A randomised-controlled trial of sulphadoxine-pyrimethamine plus artesunate versus chloroquine monotherapy for the treatment of vivax malaria

10.1 Introduction

Plasmodium vivax accounts for over half of all malaria outside Africa (Sina, 2002) and is the predominant species in south and central Asia, North Africa, Oceania and Central and South America. The majority of malaria endemic countries in south and central Asia are endemic for both *P. vivax* and *P. falciparum* and the prevailing policy has been to treat infections of either species with a standard dose of chloroquine. However, the rising and wide-spread chloroquine resistance in *Plasmodium falciparum* means that first-line treatments for falciparum and vivax malaria are having to diverge in many countries.

Often, especially at the periphery or in settings under-going or recovering from an emergency, differential malaria diagnosis may be poor. Complex emergencies are often characterized by a drain of technically qualified staff, breakdown or absence of infrastructure and non-functioning training or supervisory systems. These factors mean microscopy services, even if previously good, may be absent or poor. Although rapid diagnostic tests (RDTs) with good specificity and sensitivity are now available their stability depends on storage conditions and they are expensive. While funds are likely to be available in acute emergencies or some post-emergency settings for routine RDT use, a national diagnostic policy based on RDTs may be prohibitively expensive; no country has yet made the decision to confront the financial and logistic challenge of putting RDTs in place as routine diagnosis for a national strategy. In many settings, diagnosis remains clinical. Where microscopy is in place there is evidence suggesting that vivax malaria may be more often wrongly diagnosed as falciparum than *vice versa* (Bualombai *et al.*, 2003). Even in areas with relatively good microscopy mixed infections are often missed (Snounou and White 2004) and it appears that a mixed infection is more likely to be reported as a mono-infection of *P. falciparum* rather than a *P. vivax* because the density of *Plasmodium falciparum* is usually much higher and the gametocytes are more obvious.

It is therefore likely that incidents of vivax malaria being treated as a falciparum infection will occur on numerous occasions. It is clearly important then to know the efficacy or otherwise of the first-line falciparum treatment on vivax malaria.

There is also a need to obtain good data on the effectiveness of widely available drugs against vivax malaria. Cases of vivax malaria resistant to chloroquine, whilst currently rare, are increasing, and this may become more of a problem in the future. Confirmed reports (*in vivo*, *in vitro* and molecular) of chloroquine resistant vivax have been reported from Oceania, Asia and South America (WHO 2001b, Looareesuwan *et al.* 1999) and most recently from Peru (Ruebush *et al.*, 2003), Indonesian Papua (Sumawinata *et al.*, 2003), Turkey (Kurcer *et al.*, 2004) and Sri Lanka (Hapuarachchi, *et al.*, 2004).

In Afghanistan and Pakistan *P. vivax*, transmitted between the months of May and October, accounts for 60-90% of cases of malaria, with the remainder, transmitted between July and November being due to *P. falciparum* (Rowland *et al.* 1999). Chloroquine is thought to remain effective against *P. vivax* (Rowland and Durrani 1999), but resistance in falciparum malaria is high, with a cure rate as low as about 20% (Rab *et al.*, 2001; Durrani *et al.*, 2005; this thesis, Chapter 9). In the light of this the current recommendation of the World Health Organization (WHO) regional office is that the first-line treatment for falciparum malaria be changed to sulphadoxine-pyrimethamine plus artesunate (SP+AS) in these two countries (WHO-EM, 2004). In Afghanistan, in an environment of restructuring, rehabilitation, policy development and considerable lobbying, a change from the failing chloroquine was achieved (Kolaczinski, 2005); the MoH provisionally approved ACT (sulphadoxine-pyrimethamine plus artesunate) as the first-line treatment for *P. falciparum* (Bivigou, 2003). Although implementation of the change has been slow, it is likely to be rolled out over the next few years. In Pakistan, UNCHR has already agreed to shift to this first-line falciparum treatment in the Afghan refugee camps in the North-West of the country. In rural Afghanistan where there are limited diagnostic facilities there is a real possibility of misdiagnosis and treatment of the far more common vivax malaria, with an ACT regimen of unknown efficacy against this species.

Published data for cure-rates of vivax malaria by SP monotherapy are limited. One study puts the clinical response rate to SP at around 50% (Pukrittayakamee *et al.*, 2000; 2004), citing SP resistance as the cause. It is thought that there may be innate SP resistance of *P. vivax* associated with a sequence polymorphism at the drug-binding site of the target enzymes (Sina, 2002). However it may also be that there is a pre-existence of resistance to antifolate drugs in some areas, as a result of the use of these drugs for treatment of falciparum malaria. Mutations in the genes that encode for dihydrofolate reductase thymidylate synthetase appear to cause the vivax resistance (*dhfr* resistance) (de Pecoulas *et al.*, 1998).

Published data on the response of vivax malaria to artemisinin derivatives are more common: in one trial an equivalent fever and parasite clearance was seen following treatment with either chloroquine or a 3-day regimen of artemisinin (Phan *et al.*, 2002), other trials have shown a more rapid fever and parasite clearance following treatment with artemisinin than with chloroquine, both after a 5-day artemisinin regimen (Hamedi *et al.*, 2004; Pukrittayakamee *et al.*, 2004); and in two trials regardless of whether a 5-day or 3-day regimen of artemisinin was followed (Li *et al.*, 1994; da Silva *et al.*, 2003). However, over a 28 day follow-up more recrudescences are seen with artesunate or artemether than with chloroquine or mefloquine (Pukrittayakamee *et al.*, 2000). Only one published study has examined the use of SP+AS for vivax malaria (Tjitra *et al.*, 2002), examining the combination in an area with high rates of chloroquine resistance in both *P. falciparum* and *P. vivax* (the combination was not compared to chloroquine alone so the true chloroquine resistance rates cannot be presented). This small trial (19 of 22 patients completed to day 28) gave promising results (clinical and parasitological cure 100% at day 14 and 87.5% at day 28) showing that this combination deserves further examination for vivax malaria.

It has been noted that slowly eliminated drugs such as chloroquine and mefloquine may help prevent early relapses of vivax malaria; in South-East Asia relapses have been shown to occur as early as three weeks after the initial presentation (Pukrittayakamee *et al.*, 2004). Shorter acting drugs such as artemisinin may give rapid cure but less good results over an extended follow-up as their rapid clearance from the blood would not have the additional advantage of preventing such early relapses. Li and colleagues demonstrated a dramatic difference in relapse rates over a 9 month period with only 22% of patients treated with chloroquine relapsing, versus 79-84% of patients treated with varying doses of artemether (Li *et al.*, 1999). It is not clear what impact the combination of the rapidly-clearing artesunate with SP, a drug with a long half-life but with a possible level of *dhfr* resistance to *P. vivax*, would have on the occurrence of early relapses.

In the present study the primary aim was to demonstrate the equivalence of SP+AS with CQ in clearing parasitaemia within 7 days. The extended follow-up of 42 days was included to examine the secondary outcomes of clinical and parasitological cure over 42 days or recrudescence should there be pre-existing RI resistance to the SP+AS combination (the relatively long clearance time of SP from the blood makes an extended follow-up advisable where SP combinations are studied) or breakthrough relapses within 3-6 weeks.

One of the characteristics of *P. vivax* is its propensity to undergo early gametogenesis (Sina, 2002). Almost all symptomatic vivax patients (those presenting passively at the clinic), will be carrying gametocytes. In falciparum malaria in areas of low to moderate transmission most patients will not yet be carrying gametocytes when they present for treatment. For falciparum malaria use of drugs which kill or prevent the development of gametocytes may therefore have a substantial impact on the reservoir of parasites available for transmission, so potentially lowering the transmission of disease overall. In vivax malaria the number of patients carrying gametocytes before they present for treatment means that any potentially "transmission reducing" treatment would need to clear gametocytes as rapidly as possible. In fact, treatment of vivax malaria with chloroquine alone leads to the rapid elimination of gametocytes from the peripheral circulation (by day 4). For SP+AS to be an acceptable alternative to CQ when species diagnosis is not possible, it must demonstrate an acceptable gametocytocidal response. Artesunate has been shown to result in fewer patients carrying gametocytes after treatment than chloroquine as well as resulting in a shorter duration of carriage and gametocyte load than chloroquine (Nacher *et al.*, 2004). With SP known to result in higher numbers of *P. falciparum* gametocytes than that seen following chloroquine treatment (Targett *et al.*, 2001) it is not known what effect a combination of SP and AS will have on the gametocytes of *P. vivax*.

10.2 Materials and methods

10.2.1 Study area and population

Patient recruitment took place at the main malaria referral centre (MRC) in Jalalabad, Nangahar Province in The East of Afghanistan.

Malaria transmission in this region is seasonal and unstable. Cases of vivax malaria reach a peak in August and *P. falciparum* in October to November with transmission of both species coming to an end at the end of the year (Rowland *et al.*, 2002a). Approximately 85% of the malaria is vivax with the remainder due to *P. falciparum* (Rowland *et al.*, 2002b).

10.2.2 Patient recruitment

Patients presenting with malaria symptoms and diagnosed by microscopy as positive for vivax malaria by the routine clinic staff were referred to the study staff for further assessment. Consecutive patients were screened for the following inclusion

criteria: 1) over 2 years of age; 2) weight over 5kg; 3) not pregnant; 4) *P. vivax* mono-infection; 5) greater than 1 asexual parasite per 10 fields; 6) no other serious disease (e.g. cardiac, renal or hepatic); 7) willing to sign consent form; 8) willing to take part in a full period of follow-up; 9) no malaria drugs taken in the last 21 days; 10) no signs of severe malaria; 11) no known allergy to study drugs. All patients recruited to the study (or, in the case of children, their parents or guardians) were interviewed by the study supervisor about symptoms, previous anti-malarial therapy and use of other medications. Axillary temperature (measured underarm with an electronic thermometer) and weight were measured. A finger prick was used to take blood for a confirmatory thick and thin film.

10.2.3 Randomisation and treatment

On day 0 patients were assigned a patient number, the next consecutive number in the recruitment process. They were allocated a treatment arm from a pseudo-randomisation table sub-divided into sex and age groups. Patients were randomised to receive chloroquine ("Nivaquine", Beacon, 150mg tablets) or SP (Fansidar, Roche, 500mg/25mg tablets) plus artesunate (Plasmotrim, Mepha).

Medications were dosed according to modified weight guidelines from WHO for administration of fractions of tablets, all treatments were given orally: chloroquine, 10mg/kg day 0 and 1, 5mg/kg day 2; SP, single dose 25mg/kg sulphadoxine and 1.25mg/kg pyrimethamine on day 0; artesunate, 4mg/kg on day 0, 1 and 2.

The study supervisor was not blinded to treatment group. The patients, microscopists and health workers responsible for recording symptoms during follow-up were blinded. Study medicine was distributed and administered at the clinic and all therapy was directly observed. Patients were observed for 30 minutes after administration of medication and the dose was repeated if vomiting occurred.

10.2.4 Patient follow-up

Information and samples collected on day 0 are described above. Follow-up appointments were scheduled for days 1, 2, 3, 7, 14, 21, 28, 35 and 42. Each follow-up consultation consisted of an axillary temperature recording and completion of a standardised symptom history form. Blood was obtained by finger-prick for thick and thin films on each day, PCV on day 28. If a patient was considered to have failed on any day a PCV test was also collected on that day.

A blood spot on filter paper was also collected on day 1 and on any day of failure to be stored for PCR analysis.

Patients were encouraged to return to the clinic at any time if they felt ill. Patients presenting at the clinic on any day were examined in the same way as on a day of follow-up. Patients who did not return for a scheduled day were visited at home.

Patients were excluded after enrollment for the following reasons: 1) self-administration of additional anti-malarial drugs; 2) emergence of any concomitant febrile illness that interfered with outcome classification; 3) withdrawal of informed consent; 4) development of severe malaria or danger signs on day 0, or 5) did not present for follow-up and were not found.

The number of patients for which complete data are available does not reflect the rate of loss to follow-up. One set of raw data was lost prior to data entry, security concerns hampered the *efforts* to locate these data (see Figure 1).

10.2.5 Laboratory tests

PCV was measured with the micro-haematocrit method on day 0, day 28 and any day of failure.

Thick and thin blood smears were stained with 2% geimsa for 30 min. All slides were read by a microscopist who was blind to the treatment group and clinical outcome. Parasite density was calculated by counting the number of parasites (either trophozoites or gametocytes) per 200 white blood cells (WBC) from the thick blood smear on the assumption of a WBC count of 8000/ μ l. A smear was regarded as negative if no parasites were seen after review of 100 high-powered fields. Thin blood smears were reviewed for non-vivax infection.

One experienced senior microscopist read all slides. All slides were double read for accuracy of diagnosis (correct species, correct positive or negative result) at the HNI microscopy laboratory in Peshawar, no discrepancies were seen.

10.2.6 Sample size calculations

Published data for cure-rates for vivax malaria by SP monotherapy are limited. One small published study puts the cure rate with SP at around 50% (Pukrittayakamee, *et al.* 2000), but it was thought likely to be far better than that when combined with AS. Assuming a 99% cure rate with CQ, to detect a difference between this and an SP+AS regimen giving a cure rate of 85%, with α 0.05 and β 0.9, 91 patients would have been needed in each of the CQ and SP+AS arms. Allowing for loss to follow up of 20%, it was planned that each arm would contain 110 patients, a total of 220 patients to be enrolled.

CQ treatment leads to the rapid elimination of gametocytes from the peripheral circulation (by day 4), and has the important advantage of reducing transmission. For SP+AS to be an acceptable alternative to CQ when species diagnosis is not possible, it must demonstrate an acceptable gametocytocidal response which we consider to be <25% prevalence of gametocytes at day 7 after the start of treatment. To detect a difference with α 0.05 and β 0.9 would require the recruitment of 47 patients into each group, which is less than the number actually being proposed to be enrolled.

Logistical problems at the study site meant that only 91 patients were recruited to each arm and that some raw data were lost. Sufficient patient data on prevalence of gametocytes on day 7 and slide clearance by day 7 were available. However, the power of the study to demonstrate a difference in cure rates at day 28 is based on the 71 and 70 patients per arm for which cure data is available to day 28, with these sample sizes the power is reduced to 80%.

10.2.7 Outcome measures

The primary outcome of the study was slide clearance at day 7. In public health terms it is important that the alternate treatment demonstrates an adequate clinical response which was considered here to be 85% cure rate at 28 days. To allow a closer examination of the differences between the treatments the following secondary outcomes were also examined: clearance of both asexual and sexual parasites by day 1, outcome after day 42, proportions of patients with fever over 3 days, proportions of patients with trophozoites over 7 days and proportion of patients carrying gametocytes over the complete 42 day period, on day 7 in particular.

Patient outcomes were assessed using the WHO classifications systems for treatment of vivax malaria (WHO, 2002), these are classified as success or failure. Treatment failure is considered to have occurred for in any of the following cases:

- clinical deterioration due to *P. vivax* illness requiring hospitalization in presence of parasitaemia.
- presence of parasitaemia and axillary temperature ≥ 37.5 any time between day 3 and day 42.
- presence of parasitaemia on any day between day 7 and day 42 irrespective of clinical conditions.

Patients failing treatment in both arms were treated with chloroquine as there is no second-line treatment for vivax malaria in this region.

10.2.8 Statistical analysis

Chi squared analysis compared proportions between the two treatment groups. Risks of clinical or parasitological failure during follow-up were estimated with Kaplan-Meier survival analysis techniques.

All data were entered in Microsoft Excel (1997), the relatively small amount of data allowed for verification by checking 100% of the data once entered. Analyses were done on STATA statistical software version 6.0. A *P* value of 0.05 or less was judged significant.

Ethics

The protocol of this study was approved by the London School of Hygiene and Tropical Medicine ethical committee. Written approval to perform this trial was obtained from the Pakistan Medical Research Council.

Standard WHO methods were followed for the assessment of the efficacy of antimalarial drugs (WHO, 2002).

10.3 Results

10.3.1 Recruitment and follow-up

A total of 189 patients were randomised to the two treatment arms. Table 1 shows the baseline characteristics of patients recruited to this trial. All baseline characteristics examined were similar between the two treatment groups. All patients were carrying gametocytes on presentation. 142 patients were evaluable for 28 day outcomes. The trial profile shown in Figure 1 illustrates the loss to follow-up and loss of data. Data was available for 71 patients over the complete 42 day period.

10.3.2 Response to treatment

Using cure at day 28, as recommended by WHO protocol for assessment of vivax malaria resistance in low to moderate transmission settings (WHO, 2002), SP+AS was as effective a treatment for vivax malaria as chloroquine. A similar proportion of cases (99% and 94% respectively) responding adequately within the 28 day follow-up period. SP+AS was more effective at clearing trophozoites within 1 day of treatment than chloroquine (Table 10-3). However, by day 3, 99% of cases in both

arms were clear of asexual parasites (Figure 10-2). Fever clearance was similar between the two treatment groups (Figure 10-3)

Over an extended period of follow-up there was a divergence in the proportion of patients re-presenting with asexual parasitaemia. At the day 42 point the cumulative incidence of treatment failure was higher in the chloroquine arm than in the SP+AS arm (Figure 10-4). The proportion of patients classified as treatment failure after 42 days follow-up was significantly higher in those treated with chloroquine than in those treated with SP+AS (Table 10-2).

10.3.3 Gametocyte carriage

All patients presenting on day 0 were carrying gametocytes (Table 10-1). 99% were clear of gametocytes regardless of treatment by day 3 and none were carrying gametocytes on the 7th day after treatment (Figure 10-5). However, both on day 1 and day 2 patients treated with SP+AS were less likely to be carrying gametocytes than those treated with chloroquine.

Re-appearance of gametocytes towards the end of the follow-up period was more prevalent in the chloroquine arm, again reflecting the number of treatment failures in this arm.

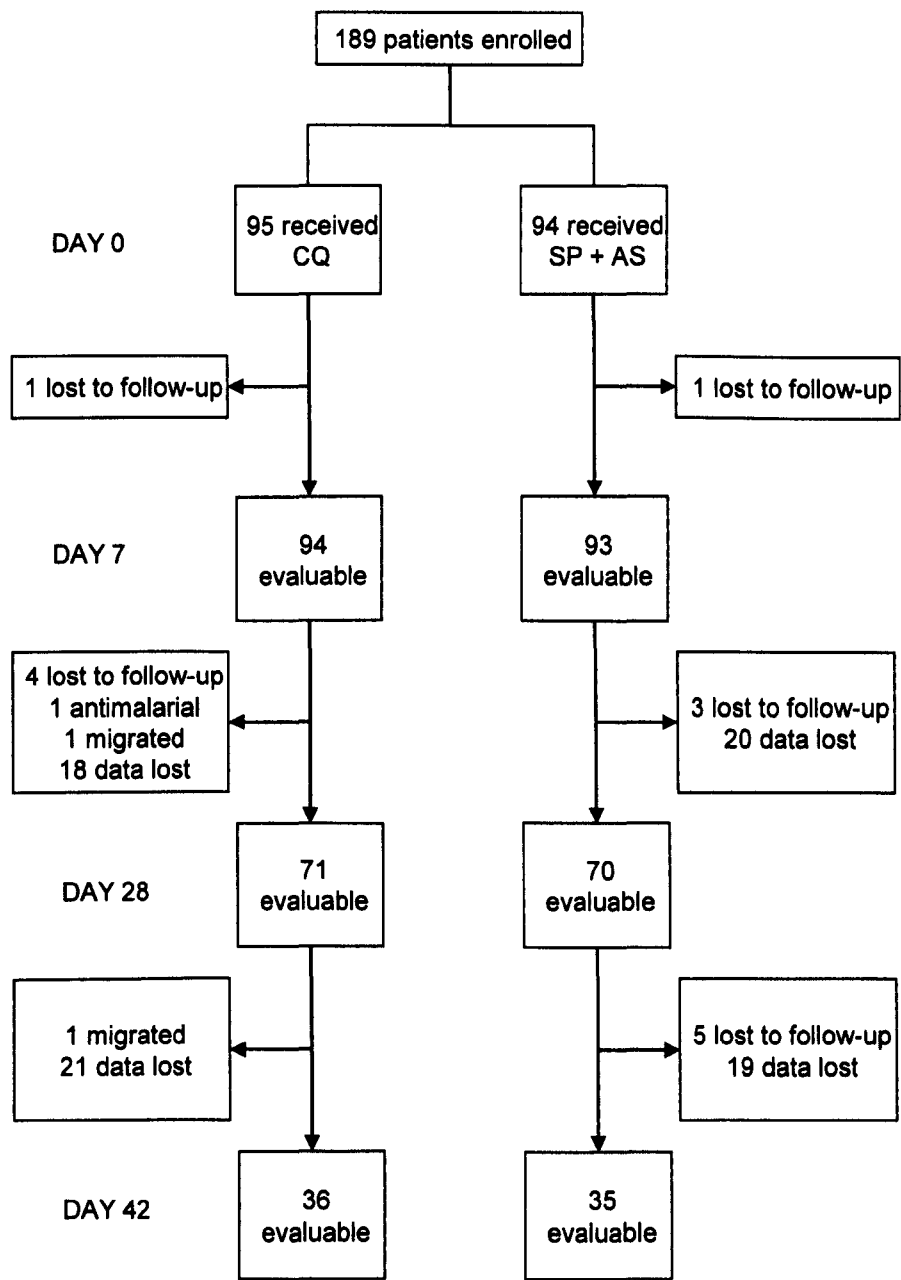


Figure 10-1. Trial profile

Table 10-1. Baseline characteristics

Variable	CQ	SP + AS
Number enrolled	95	94
Number evaluable at day 28	71	70
Age [median (IQR) years]	9 (6 - 15)	9.5 (6 - 15)
Percentage female	42	46
Weight [median (IQR) kg]	24 (17 - 45)	25.5 (16 - 44)
Temperature [mean (SD) °C]	37.0 (1.0)	37.0 (1.3)
Temperature ≥ 37.5°C on presentation [n (%)]	24 (25%)	23 (24%)
PCV [mean (SD) % haematocrit] ²	35.3 (3.4)	35.8 (3.9)
PCV < 30% [n (%)]	2 (2%)	1 (1%)
Asexual parasite density [geometric mean (95% CI) per µl]	5776 (4645 - 7132)	6621 (5402 - 8115)
Gametocyte positive [n(%)]	95 (100%)	94 (100%)
Gametocyte density [geometric mean (95% CI) per µl]	706 (559 - 891)	755 (614 - 928)

Table 10-2. Treatment outcomes at day 28 and day 42 after treatment

	CQ	SP+AS	χ^2 for outcome by arm
Number enrolled	95	94	
Percentage successfully treated (28 day follow-up), n (%)	67 (94%)	69 (99%)	$\chi^2 = 1.8224$ $P = 0.177$
Percentage successfully treated (42 day follow-up), n (%)	22 (61%)	32 (91%)	$\chi^2 = 8.9572$ $P = 0.003$

Note:

See trial profile for total numbers of patients for which data was available at day 28 and day 42.

Table 10-3. Clearance of asexual and sexual parasites

	CQ	SP+AS	χ^2 for outcome by arm
Trophozoites cleared by day 1, n (%)	69 (73%)	89 (95%)	$\chi^2 = 15.87$ $P < 0.001$
Gametocytes cleared by day 1, n (%)	38 (40%)	54 (57%)	$\chi^2 = 5.45$ $P = 0.020$
Gametocytes on or after day 3, n (%) ¹	21 (22%)	9 (10%)	$\chi^2 = 5.56$ $P = 0.018$

Note:

Of these patients 17 (81%) in the CQ arm and 4 (44%) in the SP+AS arm were later classified as treatment failures.

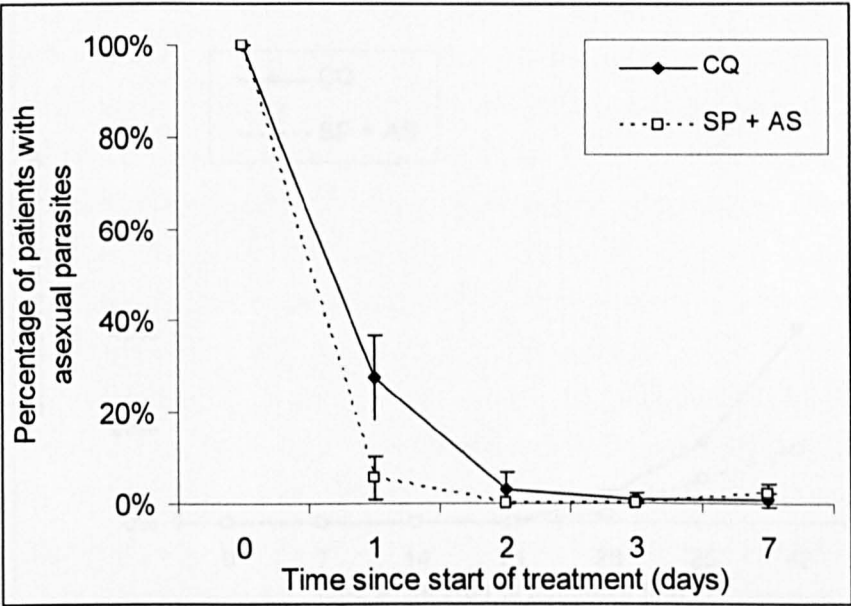


Figure 10-2. Percentage of patients carrying asexual parasites on specified days after treatment.

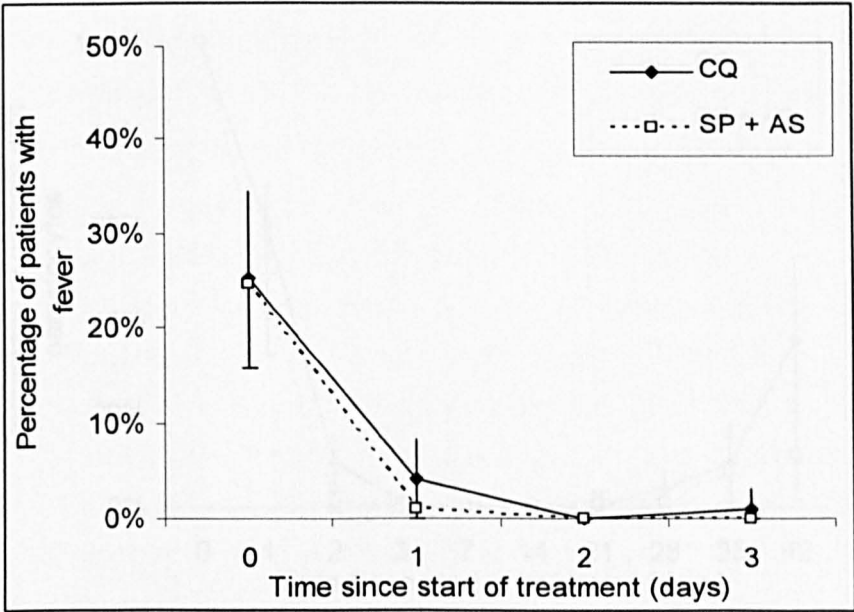


Figure 10-3. Percentage of patients with fever on specified days after treatment

Note: Error bars show the 95% confidence intervals.

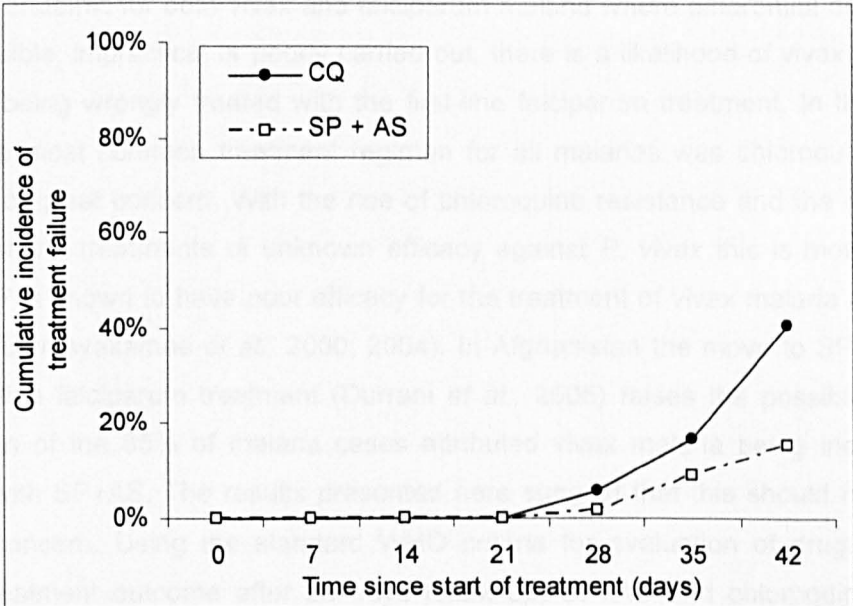


Figure 10-4. Kaplan Meier analysis of cumulative incidence of treatment failure.

Note:

Data were censored at weekly intervals, *i.e.* all those who failed in day 1-7 are classified as incident failures at day 7.

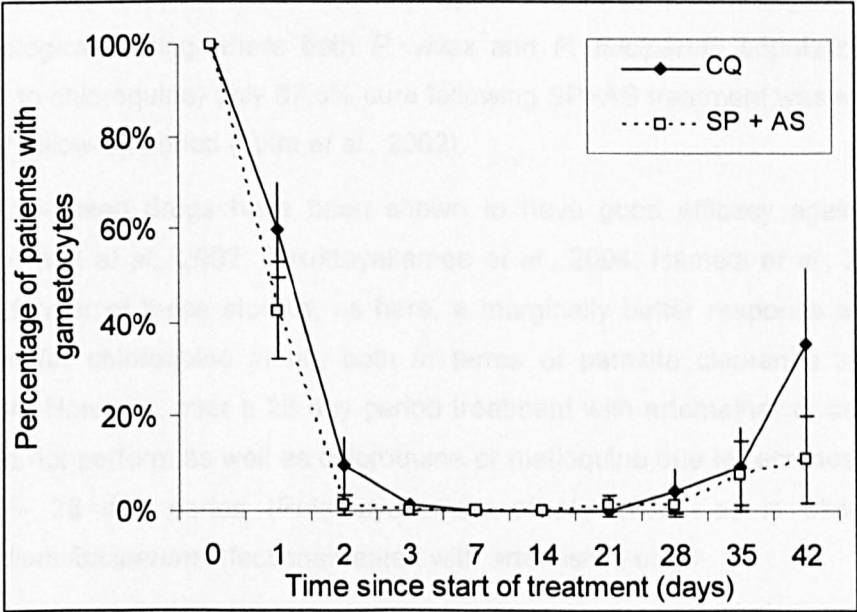


Figure 10-5. Percentage of patients carrying gametocytes on specified days after treatment.

Note: Error bars show the 95% confidence intervals.

10.4 Discussion

In areas endemic for both vivax and falciparum malaria where differential diagnosis is impossible, impractical or poorly carried out, there is a likelihood of vivax malaria patients being wrongly treated with the first-line falciparum treatment. In the past, when the most common treatment regimen for all malarias was chloroquine, this was not of great concern. With the rise of chloroquine resistance and the move to other first-line treatments of unknown efficacy against *P. vivax* this is more of an issue. SP is known to have poor efficacy for the treatment of vivax malaria in some areas (Pukrittayakamee *et al.*, 2000; 2004). In Afghanistan the move to SP+AS as the first-line falciparum treatment (Durrani *et al.*, 2005) raises the possibility of a proportion of the 85% of malaria cases attributed vivax malaria being incorrectly treated with SP+AS. The results presented here suggest that this should not be a clinical concern. Using the standard WHO criteria for evaluation of drugs for *P. vivax*, treatment outcome after 28 days follow-up, SP+AS and chloroquine were equivalent with both regimens giving excellent ($\geq 94\%$) treatment efficacy. The 99% cure of patients treated with SP+AS demonstrated here was considerably better than that seen in the only previous trial of SP+AS for the treatment of vivax malaria. In that is small study (carried out in Papua Province, Indonesia, a different epidemiological setting where both *P. vivax* and *P. falciparum* populations were resistant to chloroquine) only 87.5% cure following SP+AS treatment was seen over a 28-day follow-up period (Tjitra *et al.*, 2002).

Artemisinin-based drugs have been shown to have good efficacy against vivax malaria (Phan *et al.*, 2002; Pukrittayakamee *et al.*, 2004; Hamed *et al.*, 2004). In the two former of these studies, as here, a marginally better response was seen than that for chloroquine alone, both in terms of parasite clearance and fever resolution. However, over a 28 day period treatment with artemether or artesunate alone did not perform as well as chloroquine or mefloquine due to recrudescence in the 15 – 28 day period (Pukrittayakamee *et al.*, 2000), as is observed in *Plasmodium falciparum* infections treated with artemisinin only

The divergence of the efficacy of two treatment regimens over the period of extended follow-up is interesting but the characteristics of vivax malaria complicate the analysis of this result. In vivax malaria there are more possible causes than in falciparum malaria to account for a re-appearance of parasites. With *Plasmodium falciparum* only treatment failure (*i.e.* recrudescence) or re-infection could be the case. For vivax, recrudescence, re-infection, relapse of a previous infection or early relapse of the study infection could all be the cause of a re-appearance of parasites.

Blood spots were collected during this field-work for a parallel study of vivax population genetics in this region. PCR analysis has not yet taken place due to shortage of funds. However, such analysis can only go so far to determine the cause of re-appearance of parasites in these patients. Data demonstrating that the initial and subsequent parasitaemia were of the same parasite population would rule out re-infection or relapse of a previous infection but would not distinguish between early relapse of the current infection or treatment failure. Data demonstrating two different parasite populations were responsible for the initial and subsequent parasitaemia would rule out recrudescence or early relapse but would not distinguish between a new infection and a relapse from a previous infection.

Despite these muddled-waters some inferences can be drawn. Slowly-eliminated chloroquine is thought to be effective at preventing early relapses of vivax (Pukrittayakamee *et al.*, 2004). This would also hold true for re-infections or relapses of previous infections. The greater proportion of recurrent parasitaemia seen in the chloroquine group in the 28-42 day period may be a result either of early low-grade resistance to chloroquine in the vivax population resulting in a failure to cure the initial infection completely, or an indication that the slowly eliminated SP in the SP+AS combination provides slightly longer-term protection than chloroquine in that it suppresses relapses or reinfection during this extended period.

Whilst it was already known that patients treated with chloroquine clear gametocytes from the circulation within 2-3 days after treatment it was not clear what pattern would be seen following treatment with SP (which is known to result in a proliferation of *P. falciparum* gametocytes) in combination with artesunate (known to limit such proliferation). In fact, patients treated with SP+AS cleared gametocytes more rapidly from the circulation and the proportion of patients carrying gametocytes during follow-up was lower than those treated with chloroquine. Given that vivax patients carry gametocytes prior to presentation for treatment and that chloroquine itself clears gametocytes within the first 2-3 days it is not clear whether this small but significant difference between the two regimens would have any impact on vivax transmission. In order to determine whether this small difference would have an impact on transmission a model would have to take into account the period of time that patients are infectious to mosquitoes prior to development of symptoms and subsequent presentation for treatment. The beneficial impact of reducing early recurrent parasitaemia (when gametocytes circulate as well as trophozoites) would also need to be taken into account. Such work would be of interest; there are, however, more pressing needs in vivax research that would far

have greater impact on transmission levels, most notably the search for alternatives to the 14-day primaquine regimen that are non-toxic to G6DP-deficient patients whilst still providing radical cure.

Whilst there is no suggestion that SP+AS be considered a possible treatment of choice for chloroquine susceptible vivax malaria these data demonstrate that the efficacy of the treatment for this disease is high. In areas such as Afghanistan where SP+AS has been adopted as the first-line treatment for falciparum malaria and the likelihood of mistreatment of vivax malaria with this regimen is relatively high, the assurance can be made that vivax patients will receive adequate treatment if vivax infections are misdiagnosed as falciparum, or if mixed infections are diagnosed as falciparum only infections.

Chapter 11. General discussion and conclusions

11.1 Deciding on appropriate tools for different stages of emergencies

The accepted definitions of the “acute stage” emergency, the “post-emergency” and “chronic” stages are based on crude mortality rates and describe some of their common characteristics. However they provide no useful criteria for which intervention can be applied. More frequently than not the crude mortality rates of a given situation and the characteristics in place do not conform to these generalisations. A statement such as “intervention X is unsuitable for an acute emergency” is therefore somewhat meaningless.

In Uganda, for example, a recent mortality survey showed a crude mortality of 1.54 (95% CI 1.38 – 1.71), four times higher than the levels in unaffected areas in the same region (Uganda MoH, 2005). By the agreed definition this is therefore classed as an acute emergency. However the setting does not confirm to any of the characteristics of an acute emergency shown in Box 1-1 (Chapter 1). Most of these camps have been in existence for nearly two decades, IDPs live in mud and thatch houses similar to those found in local villages, the Ministry of Health, non-governmental organisations, UNICEF and WHO provide health care, sanitation and protection services and the services provided have moved beyond first priorities. Whilst ITNs and IRS may not be feasible tools in an acute emergency conforming to the generalised characteristics, in this setting they are certainly feasible; the IDPs live in established structures with room for erected nets and walls suitable for spraying and there are agencies and local ministry of health departments who, in collaboration would have the capacity to organise and carry out IRS campaigns or appropriately delivered ITNs with a component of health education and follow-up monitoring.

This is an example of a long-standing “acute” emergency. Often, however, acute emergencies arise because of rapid flare-ups of conflict in a chronic setting. In these cases it is also likely that many of the generalised “characteristics of an acute emergency” are not apparent. An influx of refugees or IDPs may occur resulting in some use of temporary shelters but, again, the organisations and infrastructure and stocks may be in place to enable a response with insecticide-treated nets, for example.

Regardless of the numerous types of emergency situation that will arise, it is the characteristics of each that will determine choice of intervention rather than the CMR

and the label of an “acute” or “post-” emergency. The tools tested here have potential for use in emergencies with different characteristics. Rather than specifying that, for example, insecticide-treated shelter materials are suitable for acute emergencies and long-lasting insecticide treated nets for post-emergency settings, Table 11-1 summarise some common characteristics of emergencies and indicates which control interventions may be most appropriate.

Table 11-1. Appropriate methods of controlling malaria in emergency settings.

Situation	Appropriate method of controlling malaria	Justification and constraints
<p>Recently established refugee camps (~0 – 6 months)</p>	<ul style="list-style-type: none"> • Insecticide-treated shelter materials, pre-treated if possible • After initial months assess situation and consider procuring IRS equipment and supplies or ITNs (preferably LLIN) taking lead-times for these goods into account 	<ol style="list-style-type: none"> 1. Refugees/IDPs will be living in temporary shelter 2. Organisation of spray campaigns, or net distribution problematic 3. Procurement of equipment and supplies will take time 4. Must monitor the use of the shelter material by the population to assess the likelihood of continued protection (e.g. have shelters been modified and inner walls added?)
<p>Refugees / IDPs living in permanent shelters; water is available; security does not preclude campaign style activities; ITNs are considered unsuitable (e.g. because of culture, cost, type of shelter etc)</p>	<ul style="list-style-type: none"> • IRS 	<ol style="list-style-type: none"> 1. Permanent shelters usually of mud are in use so IRS is a possible option. 2. It may have now been possible to procure equipment and supplies

Situation	Appropriate method of controlling malaria	Justification and constraints
<p>Refugees / IDPs living in permanent shelters; water is limited or security precludes campaign style activities, ITNs are considered suitable for the population</p>	<ul style="list-style-type: none"> • ITNs (preferably LLIN) delivered in a low key manner, through health facilities, local authority structures or house to house in mini-camp campaigns 	<p>1. Limited water is often a problem in camp settings where agencies may struggle to meet the SPHERE standards of minimum number of litres of water per day. An IRS campaign requires large amounts of water, mainly for the dilution of insecticide but also to ensure the spray-men can wash their uniforms and themselves at the end of each day. It may be possible to truck in water where local availability is limited but this adds considerable costs which may result in ITNs being the more affordable option.</p> <p>2. To achieve high coverage of IRS, vital for its effectiveness, there needs to be campaign style delivery with pre-campaign sensitization and mobilisation. This can be problematic in areas of poor security.</p> <p>3. ITNs are a more low key option with no need for large quantities of water and can be delivered using numerous different mechanisms depending on the situation assessment.</p> <p>4. LLINs are particularly appropriate as they will avoid the need to carry out mass re-treatment campaigns after 6 or 12 months. Any factors that make an IRS campaign unfeasible are likely to also limit the feasibility of mass net re-treatment.</p>

Situation	Appropriate method of controlling malaria	Justification and constraints
Epidemic in a refugee camp	<ul style="list-style-type: none"> • Ensure availability and use of most effective drug regimens • If possible use drug regimens that reduce gametocyte carriage e.g. ACTs • If ITNs in place, insecticide available and environment allows – ITN mass re-treatment • (If available and climate appropriate consider distribution insecticide-treated top-sheets or treatment of existing sheets used, following further development blankets may also be an option) 	<ol style="list-style-type: none"> 1. During an epidemic the first priority should always be to ensure availability of effective drugs, and ensure health staff are trained in their proper use. 2. Regimens with the potential to reduce transmission should be used if possible; ACTs appear to be the most efficacious for this. 3. Appropriate options for vector control as epidemic response are made up purely of those activities which can happen immediately. This will depend on what equipment and supplies are in stock, the skills available and a realistic assessment of how long the activity will take to organise. 4. Top-sheets treated with insecticide have been shown to be effective in some settings, this suggests insecticide-treated blankets may also be useful but the tools tested here did not show significant protection and further tool development and subsequent testing is needed.

Situation	Appropriate method of controlling malaria	Justification and constraints
<p>Environment where refugees / IDPs are beginning to return home or where migratory . nomadic populations move into insecure areas</p>	<ul style="list-style-type: none"> • LLINs • Pre-treated tents • (following more research possibly ITPS combined with LLIN) 	<p>1. LLINs are the appropriate tool to provide continued protection to returnees who move away from the scope of the existing health services or prevention activities.</p> <p>2. The evidence for the efficacy of pre-treated tents shown here and the existing data on the effectiveness indicate that pre-treated tents can provide good protection against malaria. These items could be given to populations on the move to provide protection. However, these items are heavy and bulky. This does not mean they will not be a possible option; sacks of grain or flour provided to returnees are accepted and transported for example, but the bulkiness for transport is an issue that should be considered when targeting a population on the move.</p> <p>3. ITPS may be useful to contribute to (see next point) the protection of returnees if it is used as a shelter material that has a surface available to come into contact with vector mosquitoes. If such tarpaulins would likely be used as waterproofing on the outside of a roof or to cover food or other goods the ITPS would not contribute to disease control. Further investigation on the use of such items by returnees is needed.</p> <p>4. ITPS may depend on a mass killing effect rather than prevention from biting, use as an item provided to returnees may limit the impact of this tool as the coverage in any given setting is likely to be very low. Further research is needed on the impact on disease in individual families using ITPS in combination with a method of personal protection such as an LLIN.</p>

Situation	Appropriate method of controlling malaria	Justification and constraints
Settings with low endemicity or environments where malaria epidemics are a threat	Treatment regimen which reduces gametocyte carriage	<p>1. In low transmission settings there is a high probability of regimens such as ACTs reducing transmission through the reduction of gametocyte carriage.</p> <p>2. In camp environments where epidemics are a threat such use of drugs may also be indicated.</p>

11.2 Conclusions and further research needs

11.2.1 *Insecticide treated shelter material*

Where people are living under plastic sheeting or canvas tents, insecticide-treated nets are unlikely to be a suitable option. In this case, using shelter materials that are pre-treated with insecticide or arranging a spray campaign, if equipment is available and good monitoring can be assured, are the most appropriate alternatives. The existing evidence for insecticide treated-tents as a malaria control tool (Bouma, 1996a) and results of the efficacy of insecticide-treated plastic sheeting in entomological studies suggest there is hope for a break-through in the problem of vector control for malaria in a population not living in permanent shelters and where the organisation of campaign activities are problematic.

Currently no manufacturer produces pre-treated tents commercially though the ones tested here demonstrated an impressive effect on malaria vectors with high mortality and some protection from biting. If the tents tested here were commercially available the additional benefit of the mosquito net doorway (if used properly) could enhance the level of personal protection.

The evidence to date for ITPS is encouraging, but in order to define the true usefulness of this item and the limitations to that usefulness, some considerations must be taken into account. Insecticide-treated shelters appear to provide protection from malaria in the same way as indoor-residual spraying: mosquitoes rest on the walls and roof of the shelter after (or sometimes before) taking a blood-meal and pick-up a lethal dose of insecticide leading to an overall reduction in the age of the mosquito population and the entomological inoculation rate. The protective effect is dependant on a large proportion of the shelters in an area being treated and the mosquitoes resting on these treated surfaces. The plastic sheeting distributed in emergencies provide meagre shelter and living space, minimal or no materials for erection are provided with the tarpaulins. Methods of erection therefore vary considerably as inhabitants attempt variably to maximise space, privacy or air flow depending on the environment and local culture. The entomological evaluations of the pre-treated sheeting examined structures erected in the classic ridge-pole tent format. The disease control trials underway are evaluating this sheeting as a partial or total lining for huts. How much protection will be provided by the numerous ways the sheeting will actually be used is unclear. The use of tents is more predictable as poles and ropes for construction are provided with the tent and there are seemingly few options for alternative erection techniques.

Both forms of temporary shelter will only provide protection for as long as they are being used in the way envisaged by the manufacturers and researchers. Refugee and IDP populations are seen to construct more permanent shelters, or make improvements to shelters provided (Figure 11-1), soon after arrival, perhaps within two to three months. Once internal walls or ceilings begin to be constructed the insecticide in the original shelter is no longer available to resting mosquitoes. If new whole constructions are made plastic sheeting may, for example, be relocated as waterproofing for the roof, stored for future use or sold, negating its usefulness as a vector control tool.



Figure 11-1. A “tented” refugee camp in North West Pakistan. Refugees were not allowed to build their own structures to live in. Modification of the temporary shelter available nevertheless took place.

New pre-treated shelter tools are a promising development in the options for malaria control in refugee and IDP camps. Their usefulness will focus on the early stages of an emergency when other options are not feasible. When an emergency arises, moves to get spray equipment and expertise in place should be initiated. Once the usefulness of the pre-treated shelters begins to be compromised, perhaps a few months into the emergency, it may then be possible to carry-out a spray campaign to ensure the population remains protected regardless of the types of shelter in which they are living.

11.2.2 Insecticide-treated blankets

The potential for insecticide-treated blankets as a useful malaria control tool was expected to be similar to that of insecticide-treatment of top-sheets (Rowland *et al.*, 1999). The protection from cutaneous leishmaniasis provided by insecticide-treated top sheets demonstrated previously (Reyburn *et al.*, 2000) also suggests that insecticide-treated blankets have potential that deserves further investigation. However, none of the prototype pre-treated blanket options tested here resulted in any useful reduction in blood feeding or resulted in significant levels of mortality among the vector populations. Further consideration needs to be put into the design of a pre-treated blanket. Further evaluations, with a modified testing methodology may also be useful, as well as tests carried out in settings where more anthropophilic vectors predominate. An assessment of the use of blankets in emergency environments would also be useful. In how many politically unstable settings does the climate lead to use of blankets at night? What other uses are blankets put to other than for sleeping? How often are the blankets washed? These questions, as well as further development of the tool and further entomological and epidemiological evaluations are needed.

11.1.3 Long-lasting insecticide-treated nets: conclusions and further research

PermaNet™ 2.0 and Olyset are now recommended by WHOPES as long-lasting net treatments. These tools are a great boon for malaria control, in emergency settings in particular. The data shown here demonstrated improved performance of PermaNet 2.0 over conventionally treated nets. However, the data are not directly transferable to LLINs in everyday use. Whilst it is possible to say the LLIN will remain insecticidal for considerably longer than a conventionally treated net following washing (enough information in itself to justify the use of an LLIN) it is not possible to make a statement about how long the LLIN will be efficacious in field use. This will depend on the method of washing, frequency of washing, amount of friction the net is exposed to (which may result in a wearing-off of insecticide), other environmental factors to which the net is exposed and the physical life of the net.

A key unknown in planning ITN programmes is the physical longevity of nets under different settings. Oft-cited life-spans of 5 years for polyester nets are unlikely to be realistic in most settings where nets are used all year round. Increasingly, 2-3 years is considered more likely (Kolaczinski, 2005) but there are no published data, only theorising. Simple observational but quantified studies of this would be useful in some different settings: a stable setting with seasonal malaria; a refugee camp with seasonal malaria; a stable setting with perennial malaria; a refugee camp with

perennial malaria, for example, would be useful. Although such data would be hard to extrapolate with great confidence to other settings they would be provide a useful reference point.

Appropriate methods of delivering nets in emergency settings, and the different phases of emergency would benefit from more investigation. There are numerous ways that nets are currently distributed in refugee camp settings. The vehicle for delivery: mass campaigns either house by house or using local leaders to identify recipients (e.g. families with children under five), or sustained delivery through health services to target groups (pregnant women, children attending for vaccination) are examples. The effectiveness of these strategies at reaching target audiences, and the resulting level of retention and appropriate use could be usefully reviewed; although some implementers do collect data on these parameters and report it, this information often remains in the grey literature. A thorough comparative review of the options for net delivery in a refugee camp setting would be useful.

Data that is usually not collected in the refugee setting is that of disease impact. A comparative study of different methods of net delivery would usefully include a consideration of the impact on malaria morbidity or mortality of alternate strategies such as systematically covering all households with enough nets to cover all the family as compared to covering key biologically vulnerable groups.

The potential for subsidised sale of nets in more long-term refugee or IDP settings deserves more attention which could not only examine uptake of subsidized nets but also a comparison of the effect of this system, in compared to on-going delivery of free nets, on the commercial net market.

11.2.4 Use of ACT in emergency settings

The main rationale behind the use of combination therapies for malaria is the slowing of the development of drug resistance. Whether this will prove to work in areas where the prevalence of self-treatment of fever is high (as in most of Sub-Saharan Africa (Deming *et al.*, 1989; Ejezie *et al.*, 1990; Snow *et al.*, 1992; Mnyika *et al.*, 1995; McCombie, 1996), as well as in the Indo-Pakistan sub-continent (Donnelly *et al.*, 1997a) is not clear (Hanson, 2004). Self-treatment could lead to increasing misuse of drugs and a consequent increase in selective pressure, in particular with some ACTs which have more complex drug regimens than monotherapies. Availability and use of these drugs on the open market could lead to selective pressure on the parasites of the valuable artesunate. Use of the other cheaper drugs which remain on the market may gradually erode the

appropriateness of the chosen ACT through selection of resistance to the non-artemisinin partner drug. Drugs such as Coartem®, a co-formulated regimen of artemether-lumefantrine would be useful to minimise the likelihood of poor adherence.

In a complex emergency setting other factors come into play. Self-treatment in such an environment may be less prevalent than in a stable setting: the displaced population may have less of a disposable income; there may be limited or no availability of private drug outlets, especially for a population living in a camp environment. However adherence to drug regimens dispensed by the public facilities during the acute emergency may be less good than in a stable setting: overburdened health staff may be less conscientious in supervision of treatment or in explanation of the regimen to be followed and a population under stress may behave differently from a stable setting. Again, a co-formulated regimen such as Coartem® would be useful.

Questions around the true usefulness of combinations to prevent resistance development are important for the development of national drug policies. In a refugee setting, where the onus is on using the most efficacious drug and the hope is that this situation will not be permanent queries about the true impact on resistance development should not influence the decision about whether to use ACTs, though of course other factors may influence this decision.

It is improbable that the effects of reduced gametocyte carriage would affect overall transmission levels in highly endemic settings (such as much of lowland Sub-Saharan Africa) where there is a constant reservoir of parasites in the asymptotically infected population. In the study area of low transmission it is more likely that this effect would be seen. In epidemic prone camp environments in high transmission areas use of gametocytocidal drug regimens may be effective at maintaining transmission at non-epidemic levels. Mathematical modelling of transmission trends based on the known effects of different regimens, time taken for patients to seek treatment, the level of adherence to various regimens, and the number of asymptomatic patients, would be useful to make a more informed assessment of the true impact of drug regimens known to reduce the number of circulating gametocytes.

11.3 Overall conclusion

The data presented show that there are now tools available that could make a significant contribution to the malaria control armoury in emergency settings. Some tools have the potential to provide good malaria control alone and a combination of some of these may be even more effective, for others, further work is needed in the development process.

In operational settings malaria control is almost always going to be a result of a combination of interventions, most often one vector control strategy in addition to the provision of effective treatment. Using two vector control tools, for example one providing a mass effect and one providing personal protection, in combination with the most efficacious treatment regimen available may result in a more dramatic impact on the burden of malaria.

There is a real need to test these tools, both singly and in combination, in operational settings. The difficulties of running a randomised trial in groups of camps and the good existing evidence justify taking some of these tools into operational use with concurrent evaluation.

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Annexe 1. Ethical clearance documents

**LONDON SCHOOL OF HYGIENE
& TROPICAL MEDICINE**

ETHICS COMMITTEE

APPROVAL FORM

Application number: 721



Name of Principal Investigator Mark Rowland

Department Infectious and Tropical Diseases

Head of Department Professor Pater Smith

Title Development of insecticide treated plastic sheeting for prevention and control of malaria in new refugee camps and for refugees repatriating to their homeland.

Approval of this study is granted by the Committee.

Chair

(Professor Geoffrey Targett, Acting Dean)

Date

11 October 2000

Comments from the Committee:

Wording on the consent forms need to be simplified.

Approval is dependent on local ethical approval having been received.

**LONDON SCHOOL OF HYGIENE
& TROPICAL MEDICINE**

ETHICS COMMITTEE



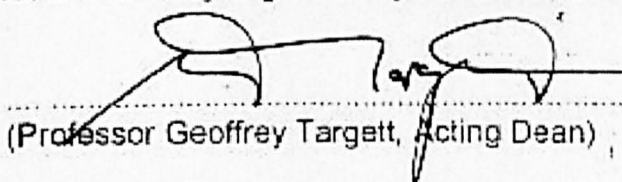
APPROVAL FORM

Application number: 650

Name of Principal Investigator **Mark Rowland**
Department **Infectious and Tropical Diseases**
Head of Department **Professor Peter Smith**

Title **Studies on combination therapy with artesunate to delay the selection of resistance to antimalarial drugs in Pakistan.**

Approval of this study is granted by the Committee.

Chair 
(Professor Geoffrey Targett, Acting Dean)

Date 6/6/00

Comments from the Committee:

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the consent form must be re-submitted to the Committee.

Pakistan Medical Research Council

Shahrah-e-Jamhuriat, Off. Constitution Avenue, Sector G-5/2, Islamabad

Ref. No. 474/2000/RDC/2633



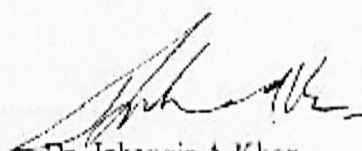
Date: 14-3-2000

ETHICS COMMITTEE APPROVAL

Subject: Ethical Clearance for the Project titled, "Randomized, double-blind, placebo-controlled study of the tolerability and efficacy of a) Artesunate plus chloroquine or sulphadoxine-Pyramethamine combinations vs (b) Single-agent chloroquine or sulphadoxine-Pyramethamine vs (c) Primaquine plus chloroquine or sulphadoxine-Pyramethamine for the treatment of uncomplicated *Falciparum* malaria in Pakistan".

Principal Investigator: Mark Rowland.

The Ethics Committee of the Pakistan Medical Research Council is satisfied with the scientific merits and design of this study. The proposed procedures for obtaining informed consent fulfil the required standard. Hereby, the committee grants ethical approval for this study.


Dr. Jahangir A Khan
Chairman
Ethical Committee

To;
Dr. Abdur Rab, Technical Adviser, HealthNet International, Peshawar.

**LONDON SCHOOL OF HYGIENE
& TROPICAL MEDICINE**

ETHICS COMMITTEE

APPROVAL FORM

Application number: 719



Name of Principal Investigator **Mark Rowland**
Department **Infectious and Tropical Diseases**
Head of Department **Professor Peter Smith**

Title **Entomological evaluation of PermaNet (manufacturer: Vestergaard),
a wash-resistant formulation for insecticide treated nets, under field
conditions in Pakistan.**

Approval of this study is granted by the Committee.

Chair
(Professor Andrew Haines, Dean)

Date 3 / 1 / 04

Comments from the Committee:

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the consent form must be re-submitted to the Committee.

Pakistan Medical Research Council

Shahrah-e-Jamhuriat, Off. Constitution Avenue, Sector G-5/2, Islamabad

Ref. No. 4-21/2004/RC/1313



Date: 28/11/2004

Dr. Mark Rowland
London School of Hygiene & Tropical Medicine,
London UK.

Subject: Ethical Clearance In Respect Of The Project Titled
"Phase-II Evaluation of PermaNETS
(Manufacturer: Vestegaard), Entomological Impact
Under Field Condition".

I am pleased to convey the approval of the Ethical Committee of PMRC in respect of the project titled "Phase-II Evaluation of PermaNETS (Manufacturer: Vestegaard), Entomological Impact Under Field Condition".

The study is well planned and organized in all aspects. Regarding use of human subjects, all the ethical issues have been properly considered and taken care off.

Dr. Jahangir A. Khan
Chairman, PMRC/
Chairman Ethical Committee

Copy to:

- ✓ The Director, Directorate of Malaria Control, Ministry of Health, Government of Pakistan, Islamabad.

Annexe 2. Publications from PhD work

Note on publications and authorship

Reprints of publications resulting from this thesis are included here. Most of the papers have several authors; the statement below clarifies my involvement in the work and paper writing.

Graham K, Mohammed N, Rehman H, Farhan M, Kamal M & Rowland M (2002) Comparison of three pyrethroid treatments of top-sheets for malaria control in complex emergencies: entomological and user acceptance studies in an Afghan refugee camp in Pakistan. *Medical and Veterinary Entomology* 16:199-206.

Involvement of first author: data analysis, preparation of the first draft of the paper and (following comments from other authors) finalisation of the paper.

Trial design and data collection were carried out without the involvement of the first author, the data were made available for analysis and inclusion in this PhD.

Graham K, Mohammad N, Rehman H, Nazari A, Ahmad M, Kamal M, Skovmand O, Guillet P, Allan R, Zaim M, Yates A, Lines J & Rowland M (2002) Insecticide-treated plastic tarpaulins for control of malaria vectors in refugee camps. *Medical and Veterinary Entomology* 16:404-408.

Involvement of the first author: Trial design, data input and analysis, preparation of the first draft of the paper and (following comments from other authors) finalisation of the paper.

Supervision of data collection was not possible due to the decision to evacuate all non-essential staff linked to the NGO HealthNet International, following September 11th bombings.

Graham K, Rehman H, Ahmad M, Kamal M, Khan I & Rowland M (2004). Tents pre-treated with insecticide for malaria control in refugee camps: an entomological evaluation. *Malaria Journal* 3:25.

Involvement of the first author: Trial design, data collection (insectary bioassays) and supervision of data collection (over-night platform work), data input and analysis, preparation of the first draft of the paper and (following comments from other authors) finalisation of the paper.

Graham K, Kayedi MH, Maxwell C, Kaur H, Malima R, Rehman H, Lines JD, Curtis CF & Rowland MW (2005) Multi-country trials comparing wash resistance of PermaNet ® and conventional insecticide-treated nets against anopheline and culicine mosquitoes. *Medical and Veterinary Entomology* 19: 72-83

Involvement of the first author: Pakistan trials: Trial design, data collection (insectary bioassays and HPLC analysis), supervision of data collection (overnight platform work), data input and analysis, preparation of the first draft of the paper and (following comments from other authors) finalisation of the paper.

Graham K. (2004) New tools to control malaria in refugee camps. *The Journal of The Royal Society for the Promotion of Health* 124:253-255.

Comment paper, no other authors.

Comparison of three pyrethroid treatments of top-sheets for malaria control in emergencies: entomological and user acceptance studies in an Afghan refugee camp in Pakistan

K. GRAHAM^{*†}, NASIR MOHAMMAD^{*}, HAMID REHMAN^{*},
MUSHTAQ FARHAN^{*}, MOHAMMED KAMAL^{*} and M. ROWLAND^{*†}

^{*}HealthNet International, Peshawar, Pakistan and [†]London School of Hygiene & Tropical Medicine, U.K.

Abstract. Insecticide-treated bedding materials (sheets and blankets) could be protective against vectors of malaria and leishmaniasis – especially in complex emergencies, epidemics and natural disasters where people are more likely to sleep in exposed situations. Comparison of cotton top-sheets impregnated with different pyrethroids (permethrin 500 mg/m², deltamethrin 25 mg/m² or alphacypermethrin 25 mg/m²) for effectiveness against mosquitoes (Diptera: Culicidae) was undertaken in a refugee camp in Pakistan. Predominant species encountered were *Anopheles stephensi* Liston, *An. pulcherrimus* Theobald, *An. nigerrimus* Giles, *Culex quinquefasciatus* Say, *Cx. tritaeniorhynchus* Giles and other culicine mosquitoes. All three pyrethroid treatments performed significantly better than the untreated sheets in deterrence and killing of mosquitoes. No significant differences were found between the three insecticides tested in terms of entomological effect. Washing of the treated sheets greatly reduced their effectiveness. In a user acceptance study conducted among 88 families (divided into four groups), six families complained of irritation of the skin and mucous membranes. Of these reports, one was from the placebo group (using untreated sheets) and the other five (5/22 = 23%) from families using deltamethrin-treated sheets. All families allocated to permethrin and alphacypermethrin groups declared an appreciation for the intervention and reported no side-effects. Ten of the placebo group disliked the intervention, citing no prevention of mosquito biting as the reason. Side-effects associated with deltamethrin indicate that alphacypermethrin and permethrin are more appropriate first choice insecticides for treatment of sheets and blankets.

Key words. *Anopheles*, *Culex*, adverse side-effects, alphacypermethrin, chaddars, complex emergency, conflict, cotton sheets, deltamethrin, disaster, insecticide-treated materials, itching, malaria prevention, mosquitoes, permethrin, pyrethroids, refugees, sneezing, vector control, Afghans, Pakistan.

Introduction

Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are the main methods of malaria prevention and

vector control in politically stable countries. Both methods have similarly impressive efficacy against malaria in South Asia and East Africa (Rowland, 1999; Curtis & Mnzava, 2000). In countries undergoing conflict or situations experiencing an acute emergency, the logistics and organization required to mount and execute a spray campaign make it impractical as an immediate protective measure. Implementation of ITNs is also fraught with problems (Rowland, 2001; Rowland & Nosten, 2001). The refugees or internally

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displaced people (IDP) may be living in temporary shelters unsuitable for erecting or using bednets. Effective bednet implementation also requires a considered approach plus health education, whereas with aid in acute emergencies the focus is on rapid distribution of shelter, blankets, food, water supply and sanitation. Impregnated bednets and other materials serve as a vehicle for efficient delivery of insecticide (usually a fast-acting pyrethroid) for personal and community protection against mosquitoes, sandflies, lice and other biting insects, especially to limit the risks of vector-borne disease transmission. Pyrethroid-impregnation of blankets has the potential to provide such protection without needing to deal with the extra financial, logistic and cultural issues raised by the distribution of bednets.

The use of permethrin-impregnated top-sheets and traditional chaddars (cotton cloth wraps used to sleep in) has been shown to exert deterrent and killing effects on malaria vectors in Pakistan (Rowland *et al.*, 1999). Their efficacy has been demonstrated against transmission of leishmaniasis (Reyburn *et al.*, 2000) as well as malaria (Rowland *et al.*, 1999). Therefore, permethrin-treated top-sheets are now used operationally for control of outbreaks in refugee camps and villages in Afghanistan. Thus, we recognize great potential for the use of pyrethroid-impregnated blankets as a rapid, effective and easy-to-apply vector control measure for epidemics, disasters and conflict situations almost anywhere in the world.

Until now, among the range of pyrethroids used for ITNs (Zaim *et al.*, 2000) only permethrin has been evaluated for top-sheets (Rowland *et al.*, 1999). As patent protection of pyrethroids expires, malaria control authorities become increasingly reliant on generic products (for ITNs and IRS) that may fluctuate in quality, price and availability. Recent mergers between agrochemical companies bring changes of product focus, whereby permethrin is superseded by alpha-cyano pyrethroids (e.g. alpha-cypermethrin, deltamethrin, lambda-cyhalothrin). Also, instead of the emulsion concentrate (EC) formulation, based on flammable hydrocarbons, less hazardous aqueous formulations of more potent pyrethroids have gained approval by the World Health Organization Pesticides Evaluation Scheme (WHOPES) www.who.int/ctd/html/whopes.html. For example, suspension concentrate (SC) formulations of pyrethroids are increasingly used for ITNs. Hence, it is vital to evaluate these innovative treatments on top-sheets, blankets and other materials in order to prove their efficacy and reliability for personal protection against vector-borne diseases.

As SC formulations of deltamethrin and alphacypermethrin are already used as acceptable treatments for ITNs (Zaim *et al.*, 2000), these products were selected for comparison with the standard permethrin EC on cotton sheets. At appropriate dosages, deltamethrin SC has insecticidal efficacy similar (Curtis *et al.*, 1996) or superior (Miller *et al.*, 1999) to that of permethrin EC against anopheline mosquitoes. Alphacypermethrin SC at 40 mg ai/m² was more effective than permethrin EC 500 mg ai/m² against *Anopheles gambiae* in hut trials and bioassays

(Jawara *et al.*, 1998). At lower dosages, lambda-cyhalothrin had efficacy comparable to permethrin (Miller *et al.*, 1995, 1999; Jawara *et al.*, 1998) and longer residual life than either permethrin or deltamethrin (Curtis *et al.*, 1996). However, the transient but uncomfortable nasal irritation and paraesthesia associated with use of lambda-cyhalothrin (Njunwa *et al.*, 1991; Maxwell *et al.*, 1999) indicates probable unsuitability as a blanket treatment.

Although the safety of the pyrethroids used here is well documented (Zaim *et al.*, 2000; Barlow *et al.*, 2001), the possibility of adverse side-effects must be examined when considering personal protection methods that involve close contact. Equally important is the question of user preferences. Although the tool may be proved effective entomologically, it will only be efficacious if used on a regular basis by individuals at risk from malaria. Experience of side-effects will of course influence this perception. Here a small-scale acceptance study was carried out to question users about their experiences.

Materials and methods

Study area and populations

Investigations were carried out at the HealthNet International field station in Azakhel refugee settlement. This camp is located close to Peshawar in the north-west of Pakistan and has existed for over 20 years. It is located on the banks of the Kabul river and is highly malarious, mostly due to *Plasmodium vivax* transmitted by *Anopheles stephensi* and other anophelines (Rowland *et al.*, 1999).

Impregnation of the top-sheets

Cotton sheets were impregnated using the following formulations and concentrations: permethrin ('Imperator' 25% EC: Zeneca, Fernhurst, U.K.) at 0.5 g/m², deltamethrin ('K-Othrin' 5% SC: Aventis, Frankfurt, Germany) at 25 mg/m², alphacypermethrin ('Fendona' 10% SC: American Cyanamid, Princeton, NJ, U.S.A.) at 25 mg/m², and a placebo treatment of 0.5% salt in water. Sheets were dried in the shade.

Outdoor platform bioassays

Platform bioassays were carried out following the procedure and Rowland *et al.* (1999). This experimental design emulates the man-vector contact that is experienced during the summer months in this area of Pakistan and neighbouring Afghanistan, when people sleep outdoors in their compound courtyards. Four raised platforms (elevated ~1 m from the ground and measuring about 8 m × 6 m) were the sites for these outdoor bioassays. Water-filled moats running along each edge kept off scavenging ants. The floor of each platform was covered by a white ground sheet to facilitate

the retrieval of dead mosquitoes in the morning. A large untreated trap net was erected on each platform (length 6 m \times width 5 m \times height 2.5 m), and a good seal made between the trap net and the ground sheet. In the centre of each trap net two men slept on mattresses on wooden frame beds, their covering for the night being the cotton sheets treated with one of the three pyrethroids or the placebo treatment. The men wore their normal clothing (*shalwar chemize*) beneath the sheets.

Starting at dusk and continuing until midnight, teams of collectors used aspirators to collect mosquitoes from the outside of the trap net and from an untreated net covering a cow nearby. These host-seeking mosquitoes would be intermittently released into the inside of the trap net, where there would be the possibility of them coming into contact with the sleepers and the treated sheets. The trap net ensured that the mosquitoes would be unable to leave the study site.

At dawn, dead mosquitoes were counted. Live mosquitoes were collected with aspirators and transferred to humidified holding cages, with sugar solution, for a holding period of 12 h (i.e. 24 h since the start of the previous night's experiment) before separation into dead and alive. All mosquitoes were then separated into blood-fed and unfed, into culicine and anopheline, and the anophelines identified to species.

The experiment was conducted over 16 nights. Each of the four treatments was evaluated on each of the four platforms on four separate occasions using four pairs of men in rotation.

Insectary bioassays

In order to examine the effectiveness of each insecticide under more controlled conditions and to examine the effect of washing, bioassays were carried out at our insectary at Adizai refugee settlement near Peshawar. Four cotton shirt-sleeves with sewn-in gloves were impregnated with either of the three insecticide treatments or the placebo. To conduct the bioassay the sleeve was worn as normal and the tester placed his arm inside a holding cage housing 50 laboratory-reared, unfed 2–5-day-old female *Anopheles stephensi* for 15 min. All mosquitoes were then transferred to a humidified paper cup containing sugar solution. The numbers of mosquitoes blood-fed/unfed and dead/alive were recorded immediately and again after 12 h.

Four people were used to test the sleeves, with each person testing each treatment once on different days. After the first 4-day rotation the sleeves were all washed with common bar soap (LuxTM) at 4 g/L for 2 min, rinsed for 2 min, and the rotation repeated.

Acceptance study

The popularity of the four treatments (three pyrethroids and placebo) was tested in Azakhel by 88 families divided

into four groups. All materials used as top-sheets and blankets at night in each household were impregnated with the treatment to which their family had been randomly assigned. The families were requested to use these as normal for 2 weeks and not to wash them. At the end of the 2 weeks a responsible member of each family who would be aware of all family members (usually a mother or elder girl) was questioned about the family's experiences using a structured questionnaire. Informed consent was obtained from all families recruited to the study. All study designs were approved by the Pakistan Medical Research Council, United Nations High Commissioner for Refugees, and LSHTM.

Statistical analysis

Statistical analyses were carried out in STATA 7 and EPI INFO 6. The proportion of mosquitoes dead and the proportion blood-fed from the entomological studies were arcsine transformed to normalize the variance and subjected to analysis of variance and unpaired *t*-tests. Data from the acceptance study were analysed using the Chi square test.

Results

Outdoor platform bioassays

Culicine mosquitoes were ~10 times more numerous than anophelines in Azakhel during the study period: 30 779 culicines and 3014 anophelines were caught and released. The anophelines were mostly *An. nigerrimus* (45%), *An. stephensi* (27%) and *An. pulcherrimus* Theobald (14%). The remaining 13% comprised *An. subpictus* Grassi, *An. fluviatilis* James, *An. annularis* Wulp, *An. culicifacies* Giles, *An. splendidus* Koidzumi and *An. maculatus* Theobald. The majority of culicines captured were *Culex quinquefasciatus* and *Cx. tritaeniorhynchus*, whereas the remainder were mostly *Cx. bitaeniorhynchus* Theobald and *Cx. vishnui* Theobald. An average of 528 ± 27 (mean \pm standard error, SE) mosquitoes were released per platform per night, comprising 481 ± 25 culicines and 47 ± 3.5 anophelines.

Table 1 shows the mean percentage mortality for each treatment type. Mortality at the control sites was lower for the culicines than for anophelines. Mean mortality fluctuated from 16% to 34% depending on the species considered. Figure 1 depicts the proportions blood-fed live, unfed live, blood-fed dead and unfed dead, after allowing 24 h for delayed, insecticide-induced mortality. It is evident from Fig. 1 that mortality rates were higher on the platforms with pyrethroid-impregnated sheets than on those with untreated sheets. This trend is significant for the anophelines as a genus and for *An. nigerrimus* as an individual species, but low mosquito numbers affected the significance for some species. Culicine mortality was generally lower than anopheline mortality and the effect of pyrethroids,

Table 1. Mortality of mosquitoes in the outdoor platform bioassays. Mean percentage mortalities shown in bold with 95% confidence limits shown in parentheses (calculated from the arcsine transformed data which were then back transformed for presentation). Values in the same column, not sharing a superscript letter are significantly different ($P < 0.05$ from unpaired t -test calculations). Treatment-induced mortality (Abbott corrected) shown in italics.

Net treatment	Mean % 24 h mortality					
	Culicines	All anophelines	<i>An. nigerrimus</i>	<i>An. stephensi</i>	<i>An. pulcherrimus</i>	Other anophelines
Alphacypermethrin	32^b (18–43) <i>19 (5–30)</i>	58^b (43–72) <i>41 (26–55)</i>	66^b (52–85) <i>49 (36–68)</i>	44.4^{a,b} (17–64) <i>28 (1–49)</i>	51^a (10–62) <i>30 (–11–41)</i>	57.7^b (27–79) <i>42 (12–64)</i>
Deltamethrin	34^b (18–47) <i>21 (6–35)</i>	57^b (43–74) <i>40 (26–57)</i>	68^b (46–83) <i>52 (30–67)</i>	44.2^b (25–62) <i>28 (10–46)</i>	44.2^a (16–69) <i>20 (–7–45)</i>	59.7^b (35–81) <i>45 (21–67)</i>
Permethrin	30^{a,b} (15–41) <i>17 (2–28)</i>	56^b (42–73) <i>39 (25–56)</i>	65^b (52–85) <i>47 (35–67)</i>	44.6^{a,b} (19–66) <i>29 (4–50)</i>	44.4^a (15–72) <i>20 (–9–48)</i>	58.9^b (27–84) <i>44 (13–69)</i>
None	16^a (10–20)	29^a (16–38)	33^a (15–46)	22.4^a (3–35)	30.3^a (6–48)	26.6^a (4–37)

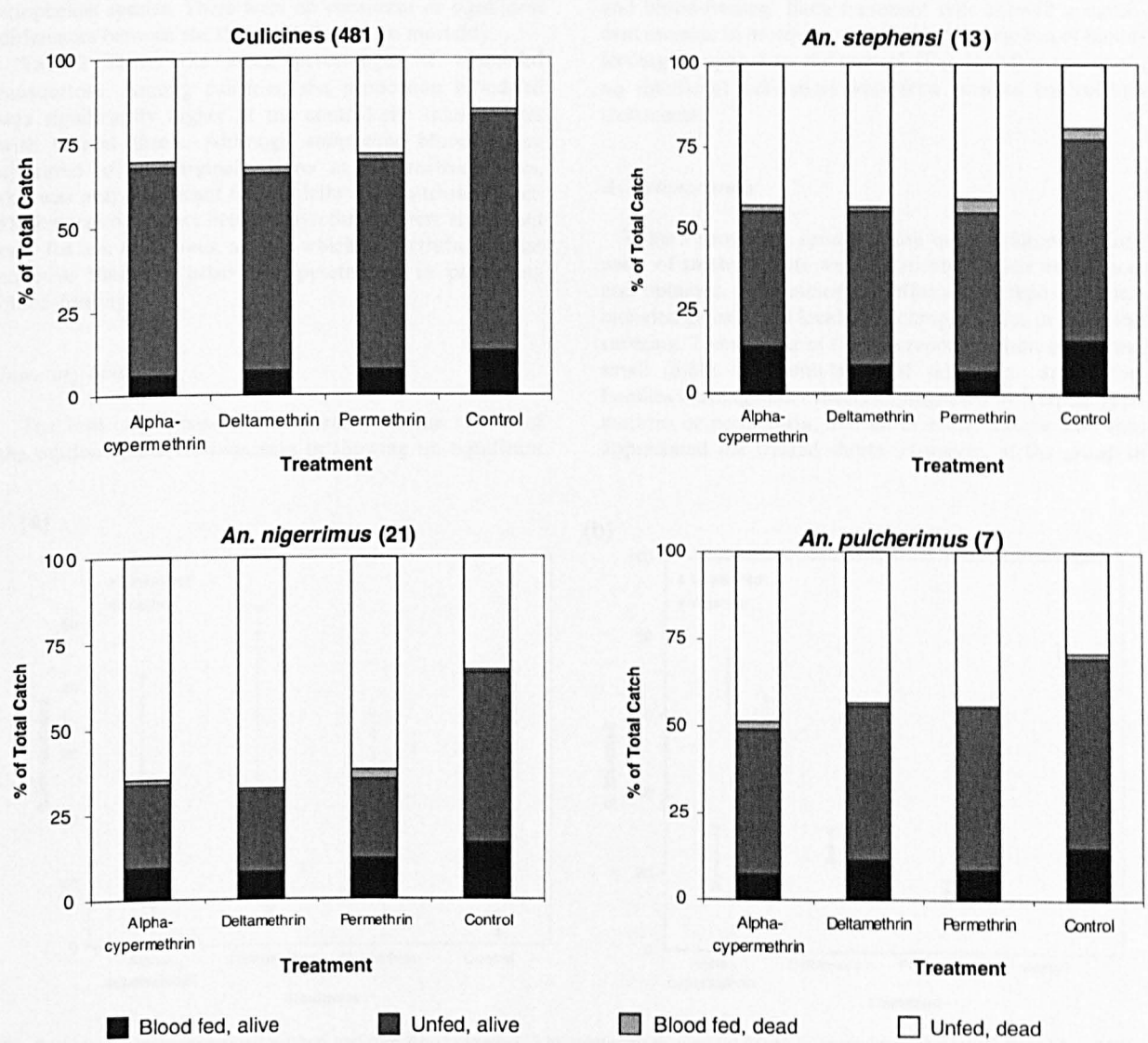


Fig. 1. Condition of mosquitoes collected from outdoor platforms. Mean number caught per platform per night shown in parentheses.

Table 2. Blood-feeding rates of mosquitoes in the outdoor platform bioassays. Mean percentages blood-fed shown in bold with 95% confidence limits shown in parentheses (calculated from the arcsine transformed data which were then back transformed for presentation). Values in the same column, not sharing a superscript letter are significantly different ($P < 0.05$ from unpaired t -test calculations). Treatment induced reduction in blood feeding shown in *italics*.

Net treatment	Mean % blood-fed					
	Culicines	All anophelines	<i>An. nigerrimus</i>	<i>An. stephensi</i>	<i>An. pulcherrimus</i>	Other anophelines
Alphacypermethrin	8.0^b (5.8–9.6) <i>45.6 (43–47)</i>	13.2^{a,b} (9.5–16.1) <i>25.8 (22–29)</i>	10.4^b (2.3–13.6) <i>39.9 (32–43)</i>	16.3^a (4.9–22.5) <i>17.3 (6–23)</i>	9.7^a (0.2–9.0) <i>41.2 (32–41)</i>	17.3^a (1.2–26.8) <i>–11.6 (–28–2)</i>
Deltamethrin	9.2^b (6.7–11.0) <i>37.4 (35–39)</i>	10.4^b (5.4–13.5) <i>41.6 (37–45)</i>	8.7^b (1.7–10.5) <i>49.7 (43–52)</i>	10.1^a (2.9–13.3) <i>48.7 (42–52)</i>	12.3^a (0.6–15.1) <i>25.5 (14–28)</i>	10.6^a (0.9–12.2) <i>31.6 (22–33)</i>
Permethrin	9.5^b (6.4–11.8) <i>35.4 (32–38)</i>	12.3^{a,b} (9.0–14.8) <i>30.9 (28–33)</i>	14.6^a (11.9–16.8) <i>15.6 (13–18)</i>	11^a (2.6–14.4) <i>44.2 (36–48)</i>	9^a (0.2–10.5) <i>45.5 (37–47)</i>	6.3^a (0.0–6.4) <i>59.4 (53–59)</i>
None	14.7^a (10.2–18.2)	17.8^a (11.4–22.3)	17.3^{a,b} (4.6–23.2)	19.7^a (5.9–26.4)	16.5^a (4.0–22.0)	15.5^a (2.6–17.7)

although significant, was not as pronounced as against some anopheline species. There were no consistent or significant differences between the three pyrethroids on mortality.

Table 2 shows the mean percentages of blood-fed mosquitoes. Among culicines, the proportion blood-fed was significantly higher at the control site than at sites with treated sheets. Although anopheline blood-feeding appeared to be marginally lower at the treatment sites, this was only significant for the deltamethrin-treated sheet. Differences of impact between pyrethroids were significant only for *An. nigerrimus*, against which permethrin was less effective than the other two pyrethroids in preventing blood-feeding.

Insectary bioassays

The insectary bioassays concurred with the results of the outdoor platform bioassays in showing no significant

difference between the three treatment types on mortality and blood-feeding. Each treatment type showed a significant increase in mosquito mortality and reduction of blood-feeding compared to the control (Fig. 2). After one wash, no significant differences were seen between control and treatments.

Acceptance study

Table 3 shows the results of the questionnaire in which users of treated sheets were asked about their experiences and opinions. Only minor side-effects were reported; these included general and localized itching, redness or rash, and sneezing. The number of families reporting these effects was small (6/88). No complaints of side-effects arose from families sleeping under sheets impregnated with alphacypermethrin or permethrin, and all of these families said they appreciated the treated sheets. However, in the group of

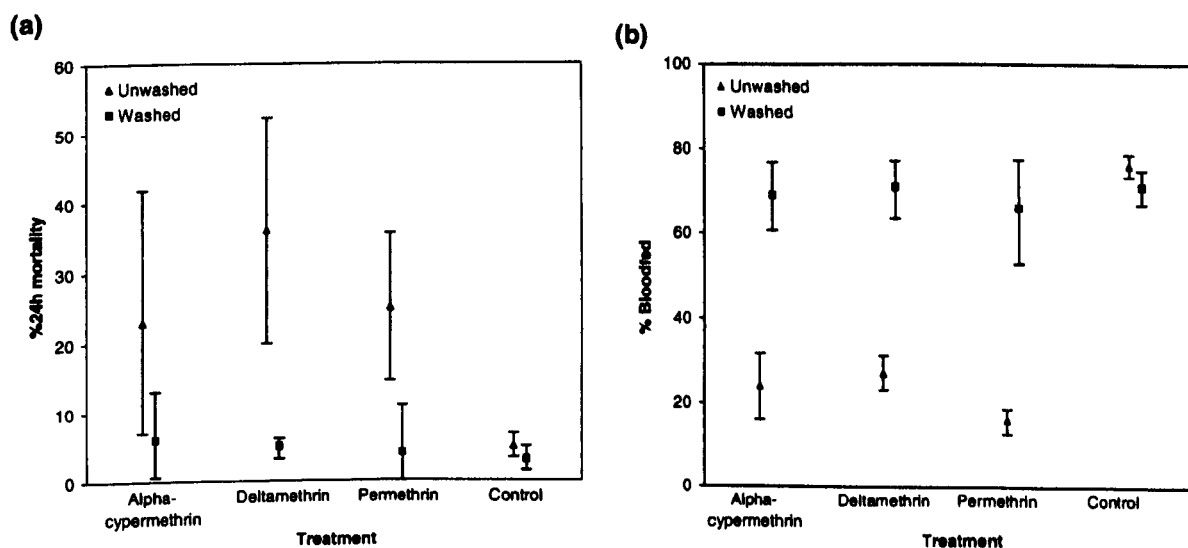


Fig. 2. Laboratory bioassays on washed and unwashed pyrethroid impregnated sleeves. (a) Mean % mortality, (b) mean % blood fed. Means and 95% confidence intervals were calculated from the arcsine transformed data and back transformed for presentation.

Table 3. Responses from users to the acceptability questionnaire. Numbers of respondents replying affirmatively when asked if they had experienced any side-effects, liked the sheets or had washed the sheets, as well as any particular side-effects mentioned when asked to specify.

	Deltamethrin (n = 21)	Permethrin (n = 24)	Alphacypermethrin (n = 22)	Placebo (n = 21)	χ^2	P-value
Any side-effects	5	0	0	1	13.05	0.0045
General itching	3	0	0	1		
Skin itching	3	0	0	1		
Eye itching	3	0	0	0		
Nose itching	2	0	0	0		
Sneezing	2	0	0	0		
Redness or rash	1	0	0	0		
Did you like it?	15	24	22	11	23.98	< 0.0001
Did you wash it?	1	2	0	0	3.42	0.3313

families using deltamethrin 5/21 families reported various minor side-effects and six families said they disliked the treated sheets. In the placebo group, one family reported side-effects (general skin itching) and only 52% stated that they liked the intervention.

Discussion

No consistent significant difference was seen in this evaluation between the insecticidal efficacy of the three pyrethroids tested, either in outdoor platform bioassays with wild mosquitoes, or in indoor bioassays with insectary-reared mosquitoes. This is an encouraging result, as it indicates that this protection method is adaptable in terms of the type of insecticide used, with both alphacypermethrin and deltamethrin achieving similar results to those previously demonstrated for permethrin (Rowland *et al.*, 1999). It had been shown previously that sleeping under sheets impregnated with permethrin can provide 64% (CI = 35–80%) protective efficacy against *Plasmodium falciparum* and 38% (CI = 0–64%) protection against *P. vivax* amongst children and teenagers (Rowland *et al.*, 1999). The comparable performance of alphacypermethrin and deltamethrin to permethrin suggests that these may also have potential as treatments for blankets. The sudden loss of insecticidal efficacy after just one wash was disappointing. Cotton generally retains pyrethroids less effectively than polyethylene or polyester (Luo Dapeng *et al.* 1994). If this control tool is to be effective operationally, it is extremely important that a pyrethroid is used which does not produce irritant effects, as this may prompt users to wash the sheets.

Adverse side-effects have been reported more often from deltamethrin use than from alphacypermethrin or permethrin use (Sexton, 1994), and this was borne out in our study. No complaints of side-effects arose from families using the permethrin or the alphacypermethrin sheets, yet a significant number did complain of irritation when using the deltamethrin treatment. Of the three respondents who reported having washed their sheets (despite being asked not to), two of these had been allocated to the deltamethrin

group and had reported side effects. The possibility that it was the irritation caused by the insecticide that prompted the users to wash the sheets is a serious concern. It is worrying to note that the nine users of deltamethrin who stated that they disliked the treated blankets gave the reason for this as being 'no noticeable prevention in mosquito biting'. This statement contradicts the entomological findings. It is a possibility that others of the deltamethrin group were also prompted by the uncomfortable side effects to wash their sheets but failed to admit to it. This may have been the cause of the surprising number of deltamethrin users perceiving the sheets as ineffective at repelling or killing mosquitoes.

In contrast to the deltamethrin and placebo users, all families using the permethrin and alphacypermethrin treatments said they liked the impregnated sheets, citing a reduction in mosquito biting as the reason. It is generally considered that methods of personal protection must have a significant effect against the more numerous, nuisance culicines in order to be appreciated by users (Guillet *et al.*, 2001). Here, the impregnated sheets did have a significant effect on culicine mortality and feeding rates, although, as is generally the case with pyrethroids (Curtis *et al.*, 1996; Maxwell *et al.*, 1999) it was a less substantial effect than against anophelines. It is encouraging that, despite this, users were able to notice a beneficial effect – suggesting that when implemented operationally it would be well used.

In addition to reducing malaria transmission and providing some protection from nuisance mosquito biting, a control tool such as this should also be successful in reducing the transmission of cutaneous leishmaniasis (CL) by phlebotomine sandflies (Diptera: Psychodidae). Permethrin-impregnated materials are impressive for reducing sandfly densities indoors: *Sergentomyia* spp. (Majori *et al.*, 1989), *Phlebotomus papatasi* Scopli (Elnaiem *et al.*, 1999) and *Phlebotomus perfiliewi* Parrot (Maroli & Lane, 1987). Permethrin-treated top-sheets can be just as effective as ITNs for protection against leishmaniasis (both achieving about 65% protective efficacy), as demonstrated in a randomised household trial in Kabul (Reyburn *et al.*, 2000). Further evaluations of this control method against CL, perhaps using alphacypermethrin, the most promising alternate insecticide evaluated here, are warranted.

For this entomological study, impregnated sheets were evaluated, as these are the materials more commonly used

as a night covering in Pakistan during the hot summer months when most malaria transmission occurs. Showing such good protective efficacy, this tool has exciting potential if adapted for more widespread complex emergency situations. Usually, blankets and quilts are among the first commodities to be distributed to refugees or IDPs, alongside food and materials for shelter. If blankets and quilts (made of wool/cotton/synthetic mix) were pre-impregnated with a pyrethroid insecticide before distribution, possibly using a long-lasting wash-resistant treatment (N'Guessan *et al.*, 2001), large-scale coverage for personal protection could be achieved rapidly. Blankets with insecticide treatment would undoubtedly be as effective in killing or deterring malaria vectors. Whereas washing reduces the insecticidal activity of pyrethroid-impregnated sheets, treated blankets would probably not be washed so frequently or vigorously, because blankets are bulkier, darker in colour and slower to appear dirty. Among the various insecticides suitable for impregnation of fabrics, etofenprox (a non-ester pyrethroid) has an advantageous safety profile (Zaim *et al.*, 2000) and could be most appropriate for use on blankets. Meanwhile, this paper reports certain advantages of alpha-cypermethrin (greater efficacy than permethrin and fewer adverse side-effects than deltamethrin), as an alternative treatment for top-sheets and blankets to be used for personal protection against vector-borne diseases.

Acknowledgements

We thank Dr Pierre Guillet for helpful comments on the manuscript. HealthNet International's malaria control and research programme is supported by the European Commission (DG1) and the United Nations High Commissioner for Refugees. This project was financed by grants from the World Health Organization and WHO/UNDP/World Bank Special Programme for Research and Training in Tropical Diseases (project no. 960662). M.R. is supported by the U.K. Department for International Development and the Gates Foundation. None of these agencies can accept responsibility for any information provided or views expressed.

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Accepted 17 April 2002

Insecticide-treated plastic tarpaulins for control of malaria vectors in refugee camps

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Abstract. Spraying of canvas tents with residual pyrethroid insecticide is an established method of malaria vector control in tented refugee camps. In recent years, plastic sheeting (polythene tarpaulins) has replaced canvas as the utilitarian shelter material for displaced populations in complex emergencies. Advances in technology enable polythene sheeting to be impregnated with pyrethroid during manufacture. The efficacy of such material against mosquitoes when erected as shelters under typical refugee camp conditions is unknown. Tests were undertaken with free-flying mosquitoes on entomological study platforms in an Afghan refugee camp to compare the insecticidal efficacy of plastic tarpaulin sprayed with deltamethrin on its inner surface (target dose 30 mg/m²), tarpaulin impregnated with deltamethrin (initially \geq 30 mg/m²) during manufacture, and a tent made from the factory impregnated tarpaulin material. Preliminary tests done in the laboratory with *Anopheles stephensi* Liston (Diptera: Culicidae) showed that 1-min exposure to factory-impregnated tarpaulins would give 100% mortality even after outdoor weathering in a temperate climate for 12 weeks. Outdoor platform tests with the erected materials (baited with human subjects) produced mosquito mortality rates between 86–100% for sprayed or factory-impregnated tarpaulins and tents (average \sim 40 anophelines and \sim 200 culicines/per platform/night), whereas control mortality (with untreated tarpaulin) was no more than 5%. Fewer than 20% of mosquitoes blood-fed on human subjects under either insecticide-treated or non-treated shelters. The tarpaulin shelter was a poor barrier to host-seeking mosquitoes and treatment with insecticide did not reduce the proportion blood-feeding. Even so, the deployment of insecticide-impregnated tarpaulins in refugee camps, if used by the majority of refugees, has the potential to control malaria by killing high proportions of mosquitoes and so reducing the average life expectancy of vectors (greatly reducing vectorial capacity), rather than by directly protecting refugees from mosquito bites. Mass coverage with deltamethrin-sprayed or impregnated tarpaulins or tents has strong potential for preventing malaria in displaced populations affected by conflict.

Key words. *Anopheles*, complex emergency, conflict, deltamethrin, insecticide-treated plastic sheeting, malaria control, malaria vectors, mass effect, mosquitoes, polythene, pyrethroid, tarpaulin, tents, refugee camp, Afghans, Pakistan.

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Introduction

Prevention of malaria is a major technical and operational problem in displaced populations affected by conflict. For control of malaria vectors by adulticidal treatment of their resting sites with residual insecticides (Najera, 1996), technical problems arise because conventional surfaces, such as walls and ceilings of houses, are not available for treatment in newly displaced or homeless populations, and because bednets are unsuitable for use in tented refugee camps (Bouma *et al.*, 1996a; Rowland, 1999, 2001; Rowland & Nosten, 2001). Operational problems because of conflict, breakdown of health services, insecurity, and inaccessible populations may combine to make it impossible to organize anything better than an emergency humanitarian response. In the early acute phase the priority needs are provision of food and water, sanitation and distribution of blankets and shelter material (Anon, 1997). Agencies specializing in emergency response have neither the time nor the capacity to mount a considered preventive response against malaria. Hence, anything that can be done to prevent malaria is more likely to be taken up by these logistic or humanitarian agencies if it places no extra demands on their established response package. Because blankets and shelter materials (plastic tarpaulins) are always distributed as part of the emergency response, these materials may constitute the only surfaces suitable or readily available for insecticide treatment. Previous work has shown that top-sheets and blankets treated with residual pyrethroid can be a useful tool against malaria and cutaneous leishmaniasis (Rowland *et al.*, 1999; Reyburn *et al.*, 2000). Likewise, canvas tents sprayed with pyrethroid are a proven intervention in malaria epidemics (Hewitt *et al.*, 1995; Bouma *et al.*, 1996a). In recent years plastic tarpaulins have replaced canvas tents as the favoured shelter material for refugees; this is because polythene sheeting is cheaper to make, cheaper to air-freight, and easier to stockpile (Rowland & Nosten, 2001). If this material could be pre-impregnated with insecticide, be shown to kill malaria vectors, and give protection against malaria it would have major advantages, as it would require no additional resources or organization other than those already deployed at the outset of an emergency. Hence, the global malaria control initiative, Roll Back Malaria (Nabarro, 1999), has been working with industry to develop factory-impregnated plastic sheeting (Allan, 2001; Allan & Guillet, 2002; Frandsen, 2002). The present paper describes the first evaluation made under controlled conditions in a refugee camp.

Materials and methods

Tarpaulins

The physical structure of the plastic tarpaulin is a core-weave matrix (90 µm thick) covered with two layers of laminate (each 45 µm), weighing 180 g/m². The core weave is made of high-density polythene and the laminates

of low-density polythene. The UNHCR (United Nations High Commissioner for Refugees) tarpaulin (made by Qin Gdao Gwhoa, Qingdao, China) is stained with blue dye. Vestergaard Frandsen (Kolding, Denmark) tarpaulin and tents were made of the same material, dyed white on the outside and black on the inside, and were impregnated with deltamethrin during manufacture. The core structure acts as a store for insecticide and the outer layers serve to physically and chemically protect the store and to regulate migration of insecticide to the surface. Owing to their physico-chemical properties, the laminates allow migration of insecticide, which builds up at the surface during storage. The laminates are impregnated with a low concentration of insecticide during manufacture. The concentration at the surface is a balance between the concentration in the core layer, migration and inactivation by ultraviolet light (UV). Through appropriate use of migration retarding chemicals and UV filters in the two laminates, a more constant effect at the surface is obtained. The concentration of deltamethrin during the mixing process was 45 mg/m² in the surface laminates, and the total concentration was 2 g deltamethrin per kg of tarpaulin. Chemical analysis (by M. Galoux at Gembloux, Belgium) using acetone extraction, showed that 20–30% of deltamethrin was lost in the processing, because the operational temperature for tarpaulin production is similar to the evaporation temperature of the insecticide. This overall analysis does not reveal the final distribution at the surface.

Laboratory bioassays

Bioassays were carried out at the London School of Hygiene & Tropical Medicine (LSHTM). Samples of factory-impregnated sheeting were either stored indoors or weathered outdoors on the roof of the LSHTM building for 84 days between November and January. Tests were done in WHO (1981) resistance test kits lined with the impregnated sheeting; untreated polythene was used as a control. Insecticide-susceptible females of *Anopheles stephensi* were exposed for 1 or 3 min (five replicates of 10 mosquitoes per replicate), then kept in the humidified holding chamber for 24 h with sugar solution before scoring mortality 24 h post-exposure.

Outdoor platform studies

The methodology of Hewitt *et al.* (1995) and Rowland *et al.* (1999) was used to simulate the type of outdoor contact that occurs naturally between host-seeking mosquitoes, shelters and sleepers. Giant trap nets (length 6 m × height 2 m × width 5 m) made of mosquito netting were erected above ant-proof platforms upon which were constructed λ-shaped shelters made from plastic sheeting, a ridge pole and two upright poles; the sheets were open at the ends and pegged to the floor along the edges. Within each shelter a man clothed in shalwar chemise and covered with a cotton sheet slept on a bedroll on the floor.

For the first half of the night wild, host-seeking mosquitoes, attracted to the platforms, were collected from the outside of the trap nets and released within. Numbers were supplemented with mosquitoes attracted to calves enclosed nearby within mosquito nets. The following morning mosquitoes were collected from the floor sheets and inner surface of the trap net, separated into dead or alive, and kept in humidified cups with sugar solution for a further 12 h before scoring delayed mortality. All mosquitoes were categorized as blood-fed or unfed, identified to genera and the anophelines to species.

The factory-impregnated plastic tarpaulin was tested against (a) a standard untreated UNHCR plastic tarpaulin as a control, (b) a UNHCR tarpaulin sprayed with deltamethrin on the inner surface at 30 mg/m² (using a Hudson X-pertTM sprayer), and (c) a tent made from the factory-impregnated deltamethrin tarpaulin (manufactured by Vestergaard Frandsen A/S). Shelters of each treatment type were tested for one night on each of four platforms in rotation.

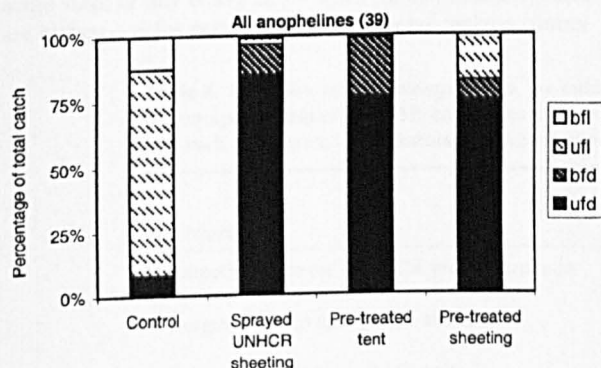
Statistical analysis

Statistical analyses were done using Stata 6 (www.stata.com). Proportional data were arcsine-transformed and subjected to analysis of variance to examine the effect of treatment on blood-feeding and mortality rates.

Results

Laboratory bioassays

On both weathered and unweathered sheeting, consistently 100% mortality of *Anopheles stephensi* females resulted from just 1-min exposure (and 24 h holding period), whereas the control mortality (on untreated sheeting) was less than 10%.



Outdoor platform bioassays

An average of 202 ± 15 (\pm standard error) culicines and 39 ± 7 anophelines were caught at each platform per night. The majority of anophelines were *An. subpictus* Grassi (18 ± 5) and *An. stephensi* (15 ± 2), plus small numbers of *An. culicifacies* Giles, *An. fluviatilis* James, *An. splendidus* Koizumi, *An. pulcherrimus* Theobald and *An. annularis* van der Wulp. With each species of anopheline present only in low numbers, the results were grouped by genera for presentation (Fig. 1). Tables 1 and 2 show the mortality and blood-feeding rates for culicines and the two most abundant anophelines. The majority of anophelines on platforms with insecticide-treated tarpaulins/tents died, whereas control mortality was never more than 6% (Culicines: $F_{3,12} = 33$, $P < 0.001$; *An. stephensi*: $F_{3,12} = 24$, $P < 0.001$; *An. subpictus*: $F_{3,12} = 46$, $P < 0.001$). There were no significant differences in mortality between the three insecticide treatments. Culicines showed slightly higher survival rates than anophelines. Blood-feeding rates were consistently low throughout the trial for anophelines and for culicines. There were no differences in blood-feeding rate between the insecticide and control treatments (Culicines: $F_{3,12} = 0.47$, $P = 0.71$; *An. stephensi*: $F_{3,12} = 0.3$, $P = 0.82$; *An. subpictus*: $F_{3,12} = 0.61$, $P = 0.62$). Figure 1 confirms that the majority of mosquitoes died unfed, presumably before making contact with the host.

Discussion

Pyrethroid-impregnated tarpaulins show good potential for malaria prevention in displaced populations. The impressive insecticidal activity demonstrated in laboratory bioassays was corroborated in field tests in the Afghan refugee camp, where contact between treated material and mosquito was near to natural. There was little effect on blood-feeding. This contrasts with the demonstration of feeding inhibition (repellency) that occurred when pyrethroid-treated top-sheets were tested on the same platforms in earlier studies (Rowland

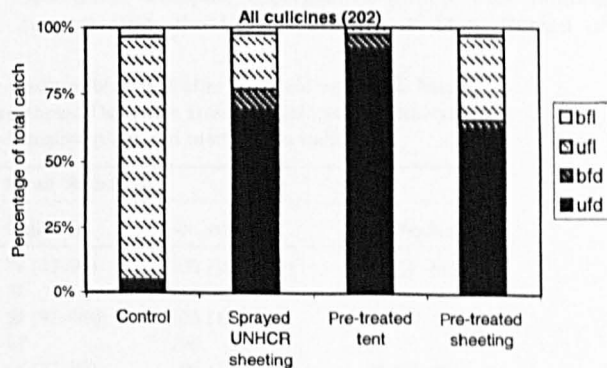


Fig. 1. Condition of mosquitoes collected from trap nets. Legend abbreviations: bf, blood-fed; uf, unfed; d, dead; l, live. Number in parentheses is the average number of mosquitoes collected per platform per night.

Table 1. Blood-feeding rates of mosquitoes in the outdoor platform bioassays. Mean percentage blood-fed with 95% confidence limits in parentheses. Data were arcsine-transformed for analysis and back-transformed for presentation.

Net treatment	Mean % bloodfed		
	Culicines	<i>An. subpictus</i>	<i>An. stephensi</i>
Deltamethrin-sprayed UNHCR plastic tarpaulin	8 (0–29)	6 (0–44)	7 (0–28)
Vestergaard factory-impregnated plastic sheeting	5 (0–16)	20 (0–77)	18 (0–78)
Vestergaard factory-impregnated plastic tent	5 (0–19)	4 (0–28)	5 (0–41)
Untreated UNHCR plastic tarpaulin (control)	3 (0–9)	6 (0–46)	11 (0–67)

et al., 1999; Graham *et al.*, 2002). Crudely erected tarpaulins offer plenty of gaps through which host-seeking mosquitoes may pass *en route* to the host. Thus, the potential of treated tarpaulins as a means of malaria prevention will depend upon generating high mortality and greatly reducing vectorial capacity, mainly by reducing survival rates among the vector population ('mass effect') rather than giving direct personal protection from biting. The prospect for disease control would remain high because coverage in new refugee camps would approach 100% as a result of free distribution of tarpaulins on registering of refugees.

An earlier evaluation of permethrin-sprayed canvas tents in Pakistan showed decay of residue within a few months of spraying of inner surfaces (Bouma *et al.*, 1996b). Better persistence was achieved with our factory-impregnated deltamethrin sheeting when weathered outdoors in London. The timing and location of this exposure (English winter) means that the sheeting would not have been subjected to particularly intense UV radiation. The UVA and B radiation, which accelerates the degradation of insecticides, is at higher levels closer to the equator and higher at comparable latitudes in the southern than in the northern hemisphere; cloud cover would also reduce UV levels. It is important that an examination of the resistance of the pre-treated sheeting to weathering also be carried out in a more severe, tropical climate. The weathering results were encouraging in showing that factory-impregnation is able to resist run-off of insecticide on exposure to frequent rain.

Although it is important that pre-treated sheeting is able to withstand weathering, this material will still be useful even if the period of residual activity lasts only a few months. The acute stage of any emergency – when human mortality rates are highest – is the period when conventional malaria control

is often thwarted by logistic and security constraints (Rowland & Nosten, 2001). Plastic tarpaulins are distributed during that initial influx of refugees. A few months later, as the camp becomes better established, refugees usually erect their own homes using locally available materials such as mud and straw. Plastic tarpaulins may be retained as useful waterproofing for roofs or walls but may also be sold on. The insecticidal activity of the tarpaulin need only last as long as IDPs and refugees are using the tarpaulins as their main shelter. Once the camp moves into a chronic stage, conventional methods of malaria control (e.g. ITN, IRS) are more easily applied.

Diarrhoeal diseases are the most important cause of mortality in refugee camps (Toole & Waldman, 1997). The significant role of houseflies in the transmission of some diarrhoeal diseases (Cohen *et al.*, 1991; Chavasse *et al.*, 1999) indicates that the potential of pyrethroid-treated sheeting to reduce housefly numbers should be examined. Leishmaniasis is another vector-borne disease that can be controlled by residual spraying (Pandya, 1983; Vioukov, 1987; Reyburn *et al.*, 2000). Insecticide-treated sheeting therefore has potential as a wider public health tool against various vector-borne diseases in refugee camps, alongside its promise as a weapon against malaria in the problematic acute phase.

Acknowledgements

HealthNet International's malaria control and research programme is supported by the European Commission. This project was supported by the Roll Back Malaria Secretariat Complex Emergencies Group with funding support from the US Department of State Bureau of

Table 2. Mortality rates of mosquitoes in the outdoor platform bioassays after 24 h holding period. Mean percentage mortalities with 95% confidence limits in parentheses. Data were arcsine-transformed for analysis and back-transformed for presentation. Abbot corrected treatment-induced mortality in italics.

Net treatment	Mean % mortality		
	Culicines	<i>An. subpictus</i>	<i>An. stephensi</i>
Deltamethrin sprayed UNHCR plastic tarpaulin	79 (45–99) <i>78</i>	100 (100–100) <i>100</i>	97 (78–98) <i>97</i>
Vestergaard pre-treated plastic sheeting	98 (93–100) <i>98</i>	100 (100–100) <i>100</i>	99 (97–100) <i>100</i>
Vestergaard pre-treated plastic tent	66 (27–96) <i>64</i>	95 (59–95) <i>94</i>	86 (43–99) <i>86</i>
Control	5 (2–10)	4 (0–33)	5 (0–23)

Population, Refugees and Migration. M.R. and J.L. are supported by the U.K. Department for International Development and the Gates Foundation. None of these donors can accept responsibility for any information provided or views expressed. For chemical analysis we are grateful to M. Galoux, Station de Phytopharmacie, Ministry of Agriculture, Gembloux, Belgium. The mention of specific companies and/or products does not in any way imply that they are recommended or endorsed by the World Health Organization in preference over others that are not mentioned.

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Accepted 22 June 2002

Research

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Tents pre-treated with insecticide for malaria control in refugee camps: an entomological evaluation

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Published: 15 July 2004

Received: 19 May 2004

Malaria Journal 2004, 3:25 doi:10.1186/1475-2875-3-25

Accepted: 15 July 2004

This article is available from: <http://www.malariajournal.com/content/3/1/25>

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Abstract

Background: A refugee shelter that is treated with insecticide during manufacture would be useful for malaria control at the acute stage of an emergency, when logistic problems, poor co-ordination and insecurity limit the options for malaria control.

Methods: Tents made of untreated canvas with deltamethrin-treated polyethylene threads interwoven through the canvas during manufacture, 'pre-treated tents', were tested in Pakistan for their impact on malaria vectors. Fixed-time contact bioassays tested the insecticidal activity of the material over 3 months of outdoor weathering. Unweathered tents were erected under large trap-nets on outdoor platforms and tested using wild-caught, host-seeking mosquitoes and insectary-reared mosquitoes released during the night into the trap-nets.

Results: The insecticide-treated tents were effective both in killing mosquitoes and reducing blood-feeding. Mean 24 hour mortality was 25.7% on untreated tents and 50.8% on treated tents ($P = 0.001$) in wild anophelines and 5.2% on untreated tents and 80.9% on treated tents ($P < 0.001$) in insectary-reared *Anopheles stephensi*. Blood-feeding of wild anophelines was reduced from 46% in the presence of an untreated tent to 9.2% ($P < 0.001$) in the presence of treated tents and from 51.1% to 22.2% ($P < 0.001$) for insectary-reared *An. stephensi*. In contact bioassays on tents weathered for three months there was 91.3% mortality after 10-minute exposure and a 24 h holding period and 83.0% mortality after 3-minute exposure and a 24 h holding period.

Conclusion: The results demonstrate the potential of these pre-treated canvas-polyethylene tents for malaria control. Further information on the persistence of the insecticide over an extended period of weathering should be gathered. Because the epidemiological evidence for the effectiveness of pyrethroid-treated tents for malaria control already exists, this technology could be readily adopted as an option for malaria control in refugee camps, provided the insecticidal effect is shown to be sufficiently persistent.

Background

Treating tents with insecticide was originally used as a malaria control tool for nomadic peoples [1]. The early work with DDT and dieldrin had little success owing to the poor adhesion of the formulations (wettable powder) then available, on fabrics [1,2]. Pyrethroid insecticides in suspension concentrate or micro-encapsulated formulations show better adhesion and residual efficacy and are more suitable for treatment of textiles. This has enabled successful treatment of tents in recent years.

Entomological evaluations have shown that canvas tents sprayed with pyrethroids can reduce mosquito feeding and cause high knock-down and mortality [3,4]. Tents sprayed with permethrin and deltamethrin reduced *Anopheles stephensi* biting by about 40% and resulted in 75% mortality amongst the blood-fed mosquitoes [5]. The ability of permethrin-treated tents to control malaria was demonstrated when they were implemented in response to an epidemic in a population of nomadic Afghan refugees in Pakistan [6].

The evidence, both entomological and epidemiological, for pyrethroid-sprayed tents being an appropriate tool for malaria control in refugee camps has led to this technology being included in several refugee health care manuals [7-9]. In recent years they have been implemented as a control method in numerous refugee and IDP settings (e.g. Sierra Leone and Mozambique [10]).

It is at the early acute stage of an emergency, when refugee camps are first being established, that poor sanitation, malnutrition and mortality due to disease are at their worst and the environment is particularly suitable for transmission of vector-borne diseases. Conventional responses to malaria control may be difficult due to insecurity, inaccessibility and inadequate inter-agency co-ordination. Organisation and implementation of insecticide spray campaigns is logistically demanding and may not be feasible at this stage. Logistical efforts are more likely to be focused on the delivery of emergency food, medicine, clean water, blankets and shelter.

If the shelter materials that are distributed during camp construction were pre-treated, a potentially effective vector control tool could be delivered with no extra demand on logistical resources.

Pre-treated polyethylene laminated tarpaulins (Zero-fly[®]) and tents (both Vestergaard Frandsen A/S), have already been demonstrated to cause mortality and reduce blood-feeding of malaria vectors [11]. Untreated plastic tarpaulins, similar to those tested, are frequently distributed to refugees. As an alternative or an addition to plastic tarpaulins, canvas tents are often distributed. Tents made com-

pletely of plastic sheeting are unlikely to be suitable as shelter materials as the conditions inside the shelter are considerably hotter and more humid than the canvas tents usually distributed in a refugee camp (Graham & Rowland unpublished data). To address these issues a canvas tent incorporating pre-treated deltamethrin-impregnated polyethylene fibres has been developed.

An entomological evaluation of these tents in Afghan refugee camps in Pakistan is presented here.

Methods

Study location

HealthNet International (HNI) maintains a working insectary and testing site at one of their field stations, the Adizai refugee settlement. The insectary rears a fully susceptible strain of *An. stephensi*. The conditions are maintained at 26 \pm 2°C and 75 \pm 10% RH. Contact bioassays and overnight platform trials with insectary-reared mosquitoes took place at this site.

Overnight platform trials with wild-caught mosquitoes took place at the entomological field station in Azakhel refugee settlement. Both sites are located on the banks of the Kabul River, approximately 25 km from Peshawar. The camps have existed for 22 years.

The land in Azakhel is waterlogged and the rise in the water table during the spring snow melt and summer monsoon gives rise to innumerable mosquito breeding sites. Mosquito populations begin to rise in April with the majority being culicine species; anopheline densities increase from July. Peak mosquito density occurs in August and density declines in November. Cases of Vivax malaria occur from March to November and Falciparum malaria from August to December. Constructed on the Azakhel site are ten elevated platforms each measuring 6 m \times 5 m and surrounded by water-filled 'moats' to exclude any scavenging ants.

Materials

The tents are made mostly of untreated canvas. Deltamethrin treated polyethylene threads (of the same material used to make Zero-fly[®]) are interwoven through the canvas fabric during manufacture. This composite material has a cream and blue striped appearance (figure 1). The tents have doors at both ends; each is made up of outer canvas door-flaps and inner mosquito mesh door-flaps. The mosquito mesh door-flaps are made from PermaNet[™] polyester netting, which is pre-treated with deltamethrin.

Contact bioassays

World Health Organization (WHO) plastic bioassay cones were taped to the inner surface of the tents. Non-blood fed, insectary-reared, female, susceptible, *An*

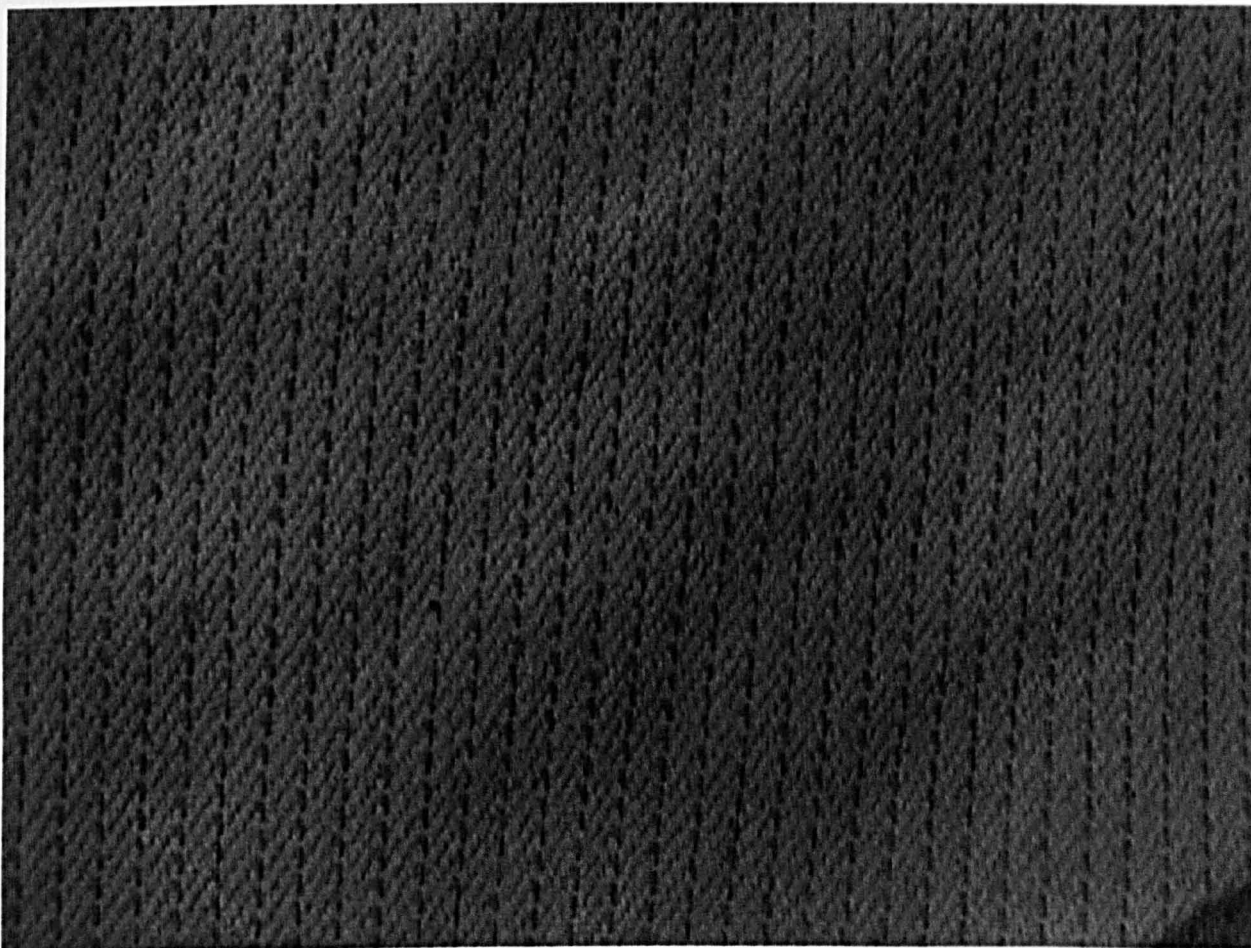


Figure 1
Close-up of tent material: canvas with delatemethrin-treated polyethelene thread woven through.

stephensi were exposed to the tent under the cones for three or ten minutes, after which they were held under insectary conditions ($26 \pm 2^\circ\text{C}$ and $75 \pm 10\%$ RH) and given access to sugar solution. Knock-down was recorded after one hour and mortality after 24 hours.

Overnight platform trials with wild-caught mosquitoes

The method of outdoor, overnight evaluation carried out at the entomological field station has been used previously to evaluate the mortality and behavioural effects of treated tents [5] treated nets [12], and treated top sheets [13,14].

Large trap-nets (length 6 m \times height 2.5 m \times width 5 m), made of untreated mosquito netting, were erected above ant-proof platforms upon which the tents were erected

(figure 2). Four men slept in each tent in local dress (cotton shalwar-chemise), each covered by a woollen blanket. At one end of the tent the doors were securely closed, at the other end the canvas door flaps were tied open whilst the mesh doors hung loose. The closed end was alternated nightly between the two ends of the tent.

For the first half of the night wild, host-seeking mosquitoes were collected from the outside of the trap-nets and released within. Near to the test site calves were tethered under untreated nets to supplement the number of mosquitoes attracted to the site and available for release within the trap-nets. The following morning all mosquitoes were collected from within the trap-nets, separated into dead or alive, and kept in humidified cups with sugar solution for a further 12 hours before scoring delayed

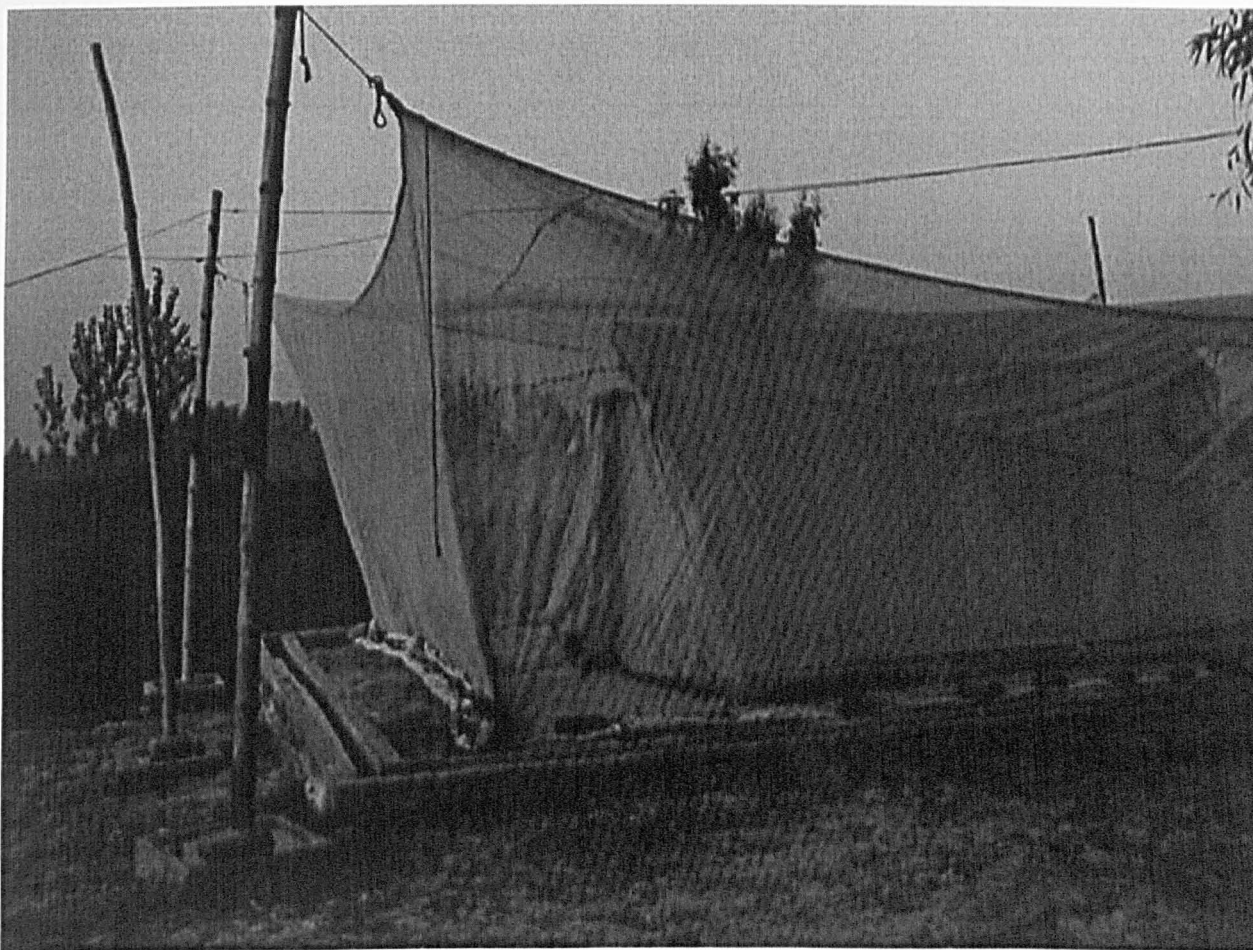


Figure 2
Tent erected in large trap net for overnight platform tests.

mortality. All mosquitoes were categorized as blood-fed or unfed, identified to genus level and the anophelines to species level.

End-point indicators used in analysis were: dawn mortality, 24 hour mortality and blood-feeding rate (feeding inhibition). Mortality analysis gives an indication of the potential mass effect on mosquito populations and blood-feeding rate an indication of personal protection. These tests took place during November 2003. All field staff gave informed consent and were given chloroquine and proguanil prophylaxis. The procedures used were approved by the Ethics Committee of the London School of Hygiene and Tropical Medicine.

Overnight platform trials with insectary-reared mosquitoes

Overnight tests were carried out with insectary-reared mosquitoes in January and February 2004, after the natural mosquito season had ended. Tents and trap-nets were erected as above. A cow was tethered within the tents in the place of sleepers. *An stephensi* and the other local vectors are highly zoophilic and cattle make a suitable alternative host.

Approximately 400 unfed, 5 – 7 day old, insectary-reared, *An stephensi* were released into the trap-nets at dusk, collected at dawn, put in holding cages with access to sugar solution and held in insectary conditions for 24 hours.

Mosquitoes were sexed and categorized as blood-fed or unfed and dead or alive. Only female mosquitoes were included in the analysis. End-points for analysis were 24 hour mortality and blood-feeding rates.

Statistical analysis

Proportional data (mortality and blood-feeding) from the platform trials and the contact bioassays were analysed using blocked logistic regression (STATA 6 software). Comparisons between treatments were made by successively dropping treatments from the overall comparison. This process allows each treatment to be compared with every other. Means and confidence limits of the constant for each treatment were back-transformed for presentation as follows:

$$x' = \frac{1}{1 + (1/\exp(x))}$$

Where: x' = back-transformed value
 x = the value from the logistic regression

Results

Overnight platform tests with wild-caught host-seeking mosquitoes in the presence of a treated tent resulted in significantly higher mosquito mortality and significantly lower blood-feeding rates than when an untreated tent was tested (table 1). The proportion killed approximately doubled for culicines ($P < 0.001$) and anophelines ($P = 0.001$), whilst blood-feeding was reduced five-fold (culicines $P = 0.001$, anophelines $P < 0.001$).

In overnight tests with insectary-reared *An. stephensi* and calves tethered inside the tents baseline mortality and blood-feeding (i.e. on the untreated tent) were higher than in the aforementioned overnight tests with wild-caught mosquitoes and human sleepers (table 2). In the presence of the deltamethrin treated tent there was a significant increase in mortality and reduction in blood-feeding of the insectary-reared mosquitoes. Mortality increased 15-fold from mortality on the untreated tent ($P < 0.001$) and blood-feeding was reduced to less than half of that seen in the presence of the untreated tent ($P < 0.001$).

Both the 3 and 10-minute exposure contact bioassays, resulted in 100% knock-down of mosquitoes within one hour of exposure (table 3). Some recovery during the holding period led to mortality being less than 100% after 24 hours in both the 3-minute and 10-minute tests (93.2% and 97.4% respectively).

After one month of weathering one hour knock-down and 24 hour mortality were greater than 95% in both the 3-minute and 10-minute tests. Some decline in insecticidal effect was seen after two and three months weathering, both knock-down and mortality after three months remained greater than 80% in 3-minute exposure bio-

assays, and greater than 90% in 10-minute exposure bioassays.

Discussion

The pre-treated canvas-polyethylene tents clearly affect mortality and blood-feeding rates of both wild and insectary-reared malaria vectors. The lower control mortality and higher treatment mortality observed with insectary-reared mosquitoes cannot be confidently assigned to genetic or behavioural consequences of insectary colonization, because of possible confounding effects due to the use of cattle as bait and testing during the cooler months of January and February (tests with wild mosquitoes were done during November using humans as bait). Whilst the magnitude of the effect differs between the trials with insectary-reared and with wild-caught mosquitoes, the conclusions to be drawn are the same.

The manufacturer of the tents tested here have combined the technology of the deltamethrin treated polyethylene (from which Zerofly® are constructed) with the accepted design of a canvas ridge-pole tent. Whilst tents made entirely of plastic sheeting were shown to be effective against malaria vectors on a previous occasion [11], tents of canvas and polyethylene may be more suitable for use in refugee camps. The superior design of these canvas-polyethylene tents was commented on by the refugee helpers involved in this trial who had also been involved in the trial of plastic tents. The sleepers reported that the canvas tents were cooler and less humid than the plastic tents (no comparative temperature or humidity data were collected during this study).

Further entomological studies are needed to assess the persistence of the insecticide. A canvas tent will be in use for longer than a plastic tarpaulin and it is, therefore, important that the residual life of the insecticide treatment is documented and, if necessary, prolonged by appropriate use of UV filters. Persistence of insecticide that gives >90% mortality in 10-minute exposure cone bioassay tests after a year of weathering would be an acceptable target. Deltamethrin has shown good persistence when sprayed on the inner surface of double-sheeted tents [5] but persistence after spraying on outer surfaces of single sheeted tents has not, to our knowledge, been examined. Outdoor weathering of the tent should continue to be monitored, with contact bioassays conducted on a monthly basis and with further overnight trials after a period of several months to one year.

Conclusions

The use of pyrethroid-treated tents is already established as a malaria control intervention. A technology that enables tents to be pre-treated with insecticide during manu-

Table 1: Blood-feeding and mortality in overnight platform trials with wild-caught mosquitoes and human sleepers.

	Number of replicates	Culicines			Number of mosq. per night Mean (SD)	Anophelines ⁴	
		Number of mosq. per night Mean (SD)	% blood fed (95% CI)	% 24 h mortality (95% CI)		% blood fed (95% CI)	% 24 h mortality (95% CI)
Insecticide-treated canvas tent	9	143 (31)	0.4%* (0.2 – 0.9)	39.4%** (36.7 – 42.1)	7.2 (4.1)	9.2%** (4.2 – 19.1)	50.8%* (38.8 – 62.6)
Untreated canvas tent	6	145 (22)	2.0%* (1.2 – 3.1)	17.1%** (14.8 – 19.8)	18.8 (18.2)	46.0%** (37.1 – 55.2)	25.7%* (18.5 – 34.5)

Notes: 1. Mean percentage blood-fed, mortality and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model. 2. Significance levels between results on the treated tent and untreated tent for each genera are indicated with asterix: * = $P = 0.001$; ** $P < 0.001$. 3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used, wild-caught mosquitoes were manually introduced to these and were then unable to leave the platform. These data are included for reference purposes only. These means do not differ significantly by t-test. 4. Due to low numbers of individual species all anophelines have been grouped.

Table 2: Blood-feeding and mortality in overnight platform trials with insectary-reared *An stephensi* and calves as bait.

	Number of replicates	Number of mosq. per night Mean (SD)	% blood fed (95% CI)	% 24 h mortality (95% CI)
Insecticide-treated canvas tent	14	257 (76)	22.2%** (20.9 – 23.6)	80.9%** (79.5 – 82.1)
Untreated canvas tent	7	262 (105)	51.1%** (48.8 – 53.4)	5.2%** (4.3 – 6.3)

Notes: 1. Mean percentage blood-fed, mortality and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model. 2. Significance levels between results on the treated tent and untreated tent for each genera are indicated with asterix: * = $P = 0.001$; ** $P < 0.001$. 3. The mean number of mosquitoes per treatment per night does not provide information about the relative attractiveness of each test item: enclosed platforms were used and one batch of insectary reared mosquitoes was released at the start of the evening (6.30–7 pm). These data are included for reference purposes only. These means do not differ significantly by t-test.

Table 3: Knock-down and 24 hour mortality in contact bioassays on the inside surface of insecticide-treated tents after weathering.

		Unweathered	1 month weathering	2 months weathering	3 months weathering
Knock-down 1 h after exposure	3-minute exposure	100% (100 – 100)	100% (100 – 100)	92.9% (85.2 – 96.8)	81.2% (74.8 – 87.8)
	10-minute exposure	100% (100 – 100)	96.3% (89.3 – 98.8)	85.2% (76.2 – 91.2)	93.0% (88.1 – 96.0)
Mortality 24 h after exposure	3-minute exposure	93.2% (84.8 – 97.2)	100% (100 – 100)	63.5% (52.8 – 73.0)	83.0% (76.7 – 87.8)
	10-minute exposure	97.4% (90.2 – 99.3)	97.6% (90.8 – 99.4)	80.7% (71.1 – 87.6)	91.3% (86.0 – 94.7)

Notes: 1. Mean percentage knock-down, mortality and 95% confidence limits are back-transformed from values calculated by the blocked logistic regression model. 2. Tests on an untreated tent were carried out as a control. 24 hr mortality was never more than 5%.

facture and be shown to retain insecticidal efficacy for up to one year would improve the feasibility of malaria control during the acute stage of an emergency.

These findings on 0 – 3 month weathered tents demonstrate that this technology is equivalent to deltamethrin

sprayed canvas tents over this period. If the criteria of adequate insecticidal persistence is met, this technology could be recommended as a good tool for malaria control in refugee camps, without the need for extensive disease control trials.

Authors' contributions

KG drafted the paper and prepared the final version, participated in the design, supervised the study and analysed the results; HR assisted in the design and supervised the field work and mosquito identification; MA assisted in study design, field work and mosquito identification; KM assisted in study design, field work and mosquito identification; IK assisted in study design, field work and identification; MR assisted in study design and revision of the paper. All authors have read and approved the final manuscript.

Acknowledgements

HealthNet International's Malaria Control Programme is funded by the United Nations High Commissioner for Refugees and the European Commission. The study was funded in part by the WHO Pesticide Evaluation Scheme and in part by Vestergaard Frandsen A/S. MR is supported by the UK Department for International Development and the Gates Foundation. None of these donors accept responsibility for any information presented or views expressed. The mention of any specific companies and/or products does not in any way imply that they are recommended or endorsed by the World Health Organization in preference over others that are not mentioned.

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Multi-country field trials comparing wash-resistance of PermaNetTM and conventional insecticide-treated nets against anopheline and culicine mosquitoes

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Abstract. Insecticide-treated bednets (ITNs) are commonly used as a means of personal protection from malaria transmission by anopheline mosquitoes (Diptera: Culicidae). Long-lasting insecticidal nets (LLINs) have special treatments intended to remain effective after many washes. The present trials assessed the efficacy and wash-resistance of several production batches of PermaNetTM (polyester net coated with polymer resin containing pyrethroid insecticide deltamethrin 55 mg ai/m²) against malaria vectors in Pakistan, Iran and Tanzania compared to ITNs conventionally treated with alphacypermethrin 15 or 20 mg ai/m², or deltamethrin 25 or 50 mg ai/m². Insecticidal efficacy of the nets before and after repeated washing (using W.H.O. recommended and traditional local washing procedures) was monitored through contact bioassays with *Anopheles* and by experimental hut and outdoor platform tests. Local washing regimes gradually reduced the insecticidal efficacy of conventionally treated nets, but they were not exhausted, even after 21 washes. Using a more rigorous laboratory washing method, insecticide was more readily stripped from conventionally treated nets. PermaNet retained high efficacy after 21 washes, giving more than 97% mortality of *Anopheles* in contact bioassays with 3-min exposure. Using the more sensitive bioassay criterion of 'median time to knockdown', PermaNet showed no loss of insecticidal activity against *Anopheles* after washing repeatedly in 2 out of 6 trials; whereas in a further three trials knockdown activity of PermaNet and conventional ITNs declined at comparable rates. Higher mortality levels of *Anopheles* in contact bioassays did not always translate to superiority in experimental hut or enclosed platform trials. In only one of four comparative field trials did PermaNet out-perform conventional ITNs after washing: this was in the trial of PermaNet 2.0 – the product with improved quality assurance. Because PermaNet and conventionally treated nets were both quite tolerant of local washing procedures, it is important in field trials to compare LLINs with conventional ITNs washed an equivalent number of times. Our comparison of PermaNet 2.0 against conventionally treated deltamethrin nets (CTDN) in Pakistan demonstrated superior performance of the LLIN after 20 washes in phase I and phase II bioassays, and this was corroborated by chemical assays of residual deltamethrin.

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Although PermaNet 2.0 has received WHOPEs *interim recommendation* for malaria control purposes, its performance should be monitored in everyday use throughout its lifespan in various cultural settings to assess its durability and long-term effectiveness for malaria prevention and control. As many millions of conventionally treated nets are already in routine use, and these will require regular re-treatment, programme strategies should be careful to preserve the effectiveness of ITNS before and after establishing the reliability of LLINs in long-term use.

Key words. *Anopheles funestus*, *An. gambiae*, *An. stephensi*, alphacypermethrin, bednets, deltamethrin, insecticide-treated nets, long-lasting insecticidal nets, malaria control, malaria vectors, PermaNetTM, pyrethroid-impregnated nets, wash-resistance, Iran, Pakistan, Tanzania.

Introduction

Insecticide-treated nets (ITNs) are commonly used to reduce the risk of malaria transmission. Periodic re-treatment of ITNs with pyrethroid insecticide is necessary for their continued effectiveness against anopheline mosquitoes (Lines, 1996). Removing the need for re-treatment would circumvent a major operational problem faced by net owners and by ITN projects based on marketing re-treatment. Some manufacturers of chemicals and textiles are developing treatment processes in which the insecticide is more stably bound to fabrics (W.H.O., 2004b), resulting in long-lasting insecticidal nets (LLINs). The aim is to develop treatments that can resist washing and that enable nets to remain efficacious for their useful lifespan of perhaps 4–5 years. The first type of LLIN recognized by the World Health Organization Pesticide Evaluation Scheme (WHOPEs) was the Olyset Net[®] (Sumitomo Chemical Co., Osaka, Japan), a wide-mesh polyethylene net with permethrin 2% w/w incorporated during manufacture (Itoh & Okuno, 1996; N'Guessan *et al.*, 2001; W.H.O., 2001; Tami *et al.*, 2004).

PermaNetTM is another type of LLIN, made of polyester treated with deltamethrin 55 mg ai/m², developed by Vestergaard Frandsen A/S (Kolding, Denmark). Field trials of PermaNet manufactured in 2000–2001 have given inconsistent bioassay results (W.H.O., 2004a), showing efficacy persisting after washing in one South American study (Gonzalez *et al.*, 2002), but showing efficacy declining with successive washes in one African study (Müller *et al.*, 2002). Therefore, during 2002 the manufacturer launched 'PermaNet 2.0' the manufacturing process remained unchanged but measures were taken to overcome the earlier variability by improving quality assurance during manufacture (W.H.O., 2004b). This study reports on evaluations of both versions of PermaNet compared to conventionally treated ITNs subjected to equivalent washing regimes. Without such controls any claim of improved wash-fastness lacks proof. Among published studies fulfilling this criterion, Gonzalez *et al.* (2002) tested PermaNet in parallel with conventional polyester ITNs of three treatment types [SC (suspension concentrate, flowable) alphacypermethrin 40 mg ai/m² or deltamethrin 25 mg ai/m² using SC and

tablet formulations], finding no difference in insecticidal efficacy after three gentle washes but, with more aggressive washing, PermaNet outperformed the conventional ITNs. Conversely, Asidi *et al.* (2004) found no improved performance of PermaNet over ITNs conventionally treated with SC alphacypermethrin 20 mg ai/m², CS (capsule suspension, microencapsulated) lambdacyhalothrin 18 mg ai/m², or EC (emulsifiable concentrate) permethrin 500 mg ai/m² after five washes and 8 months of use. To further elucidate the vital issue of PermaNet performance relative to that of conventionally treated nets under various field conditions, this study summarizes six trials in three countries that involved use of local wash procedures and evaluation by standard laboratory and field-based bioassay methods.

Materials and methods

Study sites

Study sites have been described elsewhere for Muheza-Ubwari, Tanzania (Curtis *et al.*, 1996), for Kazeroun, Iran (Kayedi, 2004) and for Peshawar, Pakistan (Hewitt *et al.*, 1995).

The trial of PermaNet 2.0 phase I bioassay tests and chemical assay of residual deltamethrin took place at the London School of Hygiene and Tropical Medicine (LSHTM) under controlled conditions of temperature and humidity.

Nets and treatments

For each field trial, the bednets used for conventional treatment had the following material specifications.

Pakistan trials: green polyester multifilament, 100 denier, mesh 156 holes/inch², dimensions 220 cm long × 150 cm high × 180 cm wide, manufactured by SiamDutch Mosquito Netting Co., Bangkok, Thailand.

Iran trial: white polyester multifilament, 100 denier, mesh 156 holes/inch², dimensions 180 cm long × 150 cm high × 130 cm wide, manufactured by Vestergaard Frandsen Disease Control Textiles, Hanoi, Vietnam.

Tanzania trials 1 and 3: green polyester multifilament, 75 denier, mesh 144 holes/inch²; dimensions 180 cm long × 150 cm high × 130 cm wide, manufactured by A to Z Textiles, Arusha, Tanzania. For the second Tanzanian trial, nets were the same as for the Iran trial.

Standard procedures were followed for the mixing of pyrethroid insecticide treatments, and the dipping and drying of nets (Chavasse *et al.*, 1999). In Pakistan, conventionally treated deltamethrin nets (CTDNs) were impregnated with dilute K-Othrin[®] 2.5% SC (AgrEvo, Berkhamsted, U.K.) to give a target dosage of deltamethrin 50 mg ai/m² (approximating the PermaNet dose) for the first trial and 25 mg ai/m² for the second trial. For the Iranian and second Tanzanian trials, CTDNs were treated with aqueous suspensions of KO-tab[®] (Aventis, Centurion, South Africa), a pre-packaged individual net treatment that gives a deltamethrin target dose of 25 mg ai/m² (W.H.O., 1999). Conventionally treated alphacypermethrin nets were impregnated with dilute Fendona[®] 10% SC (W.H.O., 1998a) giving a target dosage of 15 mg ai/m² for trials in Pakistan (supplied by BASF, Mount Olive, NJ, U.S.A.) and 20 mg ai/m² for trials in Tanzania (insecticide supplied by America Cyanamid, Gembloux, Belgium).

PermaNet is made of polyester multifilament (denier 100, mesh 156 holes/inch²), surface-treated with deltamethrin at 45–55 mg ai/m² in a resin foundation (W.H.O., 2004a). Originally made in white or green, PermaNet of both colours were included in the first Tanzanian trial, but only white PermaNets were evaluated in other trials. Samples of the earlier version of PermaNet were supplied by Vestergaard Frandsen as follows: early 2000 to Tanzania for trial 1, February 2001 to London and forwarded to Pakistan and Tanzania for trial 2, April 2001 to Iran, and April 2002 to Tanzania for trial 3. Samples of PermaNet 2.0 were received in April 2003 in Pakistan.

Washing procedures

In five of the six trials the nets were washed vigorously by hand for 2–3 min in tapwater at ambient temperature, according to local practices. In Iran the water was chlorinated, but not in Pakistan or Tanzania. The soaps and detergents used were: in Pakistan, Lux[™] soap bar (<http://www.unilever.com>) 4 g/L giving pH 7.9 solution; in Iran, Barf[™] powder (Paksun Co., Tehran) detergent; in Tanzania, local soap and Foma[™] (SDL, Tanga, Tanzania) detergent 1.8 g/L giving pH 9.75 solution.

In the second Pakistan trial, with PermaNet 2.0, the washing methods preceding phase I and II tests were different from other trials. Intact bednets destined for phase II testing were subjected to a harsher wash regime than that used previously, the nets being soaked for 60 min in water at ambient temperature, washed for 30 min in a top-loading 2-way-spin washing machine with water at 40 ± 5°C and rinsed for 15 min at ambient temperature in the washing machine. Swatches of net tested in phase I bioassays were washed using the newly established interim standard

wash practice of W.H.O. (2004a). Swatches of net (25 cm × 25 cm) were washed in 0.5 L of alkaline (pH 9) soap solution (2 g/L) in deionized water at 30°C in a water bath shaker (model SW 20: Julabo GmbH, Seelbach, Germany) at 155 strokes/min for 10 min, followed by two 10 min rinses in deionized water at 30°C, also using the water bath shaker. All nets and net swatches were dried in the shade. One set of net swatches was stored before washing and the other sets after 10, 20 and 30 washes. These were then tested in phase I bioassays in London and examined for deltamethrin content using HPLC.

Test procedures

The test methods used in the six trials are shown in Table 1.

Insecticide bioassays

Phase I assays took place at the field study sites, except in the second Pakistan trial when phase I bioassays were carried out at the LSHTM.

Insectary-reared susceptible *Anopheles stephensi* Liston were used for bioassays in Pakistan and at LSHTM (using *An. stephensi* Beech strain), whereas wild-caught mosquitoes were used elsewhere: *An. stephensi* in Iran, *An. gambiae* Giles and *An. funestus* Giles in Tanzania.

Two types of contact bioassay were undertaken, as described by W.H.O. (1998b). For time-limited exposure tests, batches of mosquitoes were tested on treated netting for 3 min, knockdown scored after 1 h and mortality after 24 h. To assess the median time to knockdown (MTKD), batches of 11 mosquitoes were exposed continuously to treated netting and the observed time to knockdown of the median (6th) mosquito was recorded. In the bioassays carried out in Pakistan or London, W.H.O. plastic bioassay cones were used to expose mosquitoes to netting in 3-min exposure tests, and the method of confining mosquitoes in a wire ball-frame with netting wrapped around was used to determine MTKD. In all other trials the wire ball-frame exposure method was used for all bioassays.

Trials of nets in experimental huts and on enclosed platforms

In Tanzania, field trials were carried out in experimental huts fitted with veranda-traps (Smith & Webley, 1969; Curtis *et al.*, 1996). Inside each hut, two volunteers slept overnight within the test net. Host-seeking wild mosquitoes (anophelines and culicines) were able to enter and exit the hut via open eaves on two sides. Eaves on the other two sides opened into screened verandas that trapped the mosquitoes exiting on those sides; there were also exit traps on the hut windows. For each hut, the numbers of mosquitoes dead and blood-fed were recorded after sunrise, and the total number of mosquitoes entering the hut, T_e , was estimated as $T_h + T_w + 2T_v$, where T_h = number of mosquitoes found in the hut,

Table 1. Test methods used in the six trials.

Trial	Phase I Bioassay method:		Phase II Field test method	HPLC assay of deltamethrin content
	% mortality 24 h after 3-min exposure	Median time to knockdown (MTKD)		
First Tanzania	Yes	Yes	Not done	No
Second Tanzania	Yes	Yes	Experimental hut	No
Third Tanzania	No	Yes	Experimental hut	No
Iran	Yes	Yes	Not done	No
First Pakistan	Yes	Yes	Enclosed platform	No
Second Pakistan	Yes	No	Enclosed platform	Yes

T_w = number of mosquitoes in the window traps and T_v = number of mosquitoes in the two screened veranda traps (the other two verandas being open).

In the Pakistan trials the test nets were erected on outdoor platforms covered by giant trap-nets (2 m high, 5 m wide and 6 m long) (Hewitt *et al.*, 1995). Two sleepers lay under each test net. During the evening, wild host-seeking female mosquitoes (anophelines and culicines) attracted to the platforms were transferred by collectors (using mouth-operated aspirators) from outside to inside the trap-nets where they would encounter the test net. In the morning, all mosquitoes were collected from inside the trap-net and held with access to sugar solution until the evening, when mortality was scored. The platform and trap-net method has been used previously to evaluate ITNs (Hewitt *et al.*, 1995), insecticide-treated tarpaulins (Graham *et al.*, 2002b) and top-sheets (Rowland *et al.*, 1999) used by people sleeping outdoors at night.

To assess mosquito blood-feeding inhibition by the net treatment, in the Tanzanian trials and the second Pakistan trial the nets were deliberately holed (six 4 × 4 cm holes) to simulate worn and torn nets.

High performance liquid chromatographic assay of deltamethrin on nets

Following the second Pakistan trial of PermaNet 2.0 and CTDN, nets that had been washed (by the washing machine method) and used for phase II overnight platform tests, plus swatches of net that had been washed (by the water bath method) were sent to the London School of Hygiene and Tropical Medicine (LSHTM) for assays of deltamethrin content by HPLC, using the Dionex Summit (Camberley, Surrey, U.K.) range of equipment and software. Deltamethrin was extracted with acetonitrile and samples were separated on an Acclaim[®] C₁₈ 120 Å, 250 × 4.6 mm column (Dionex) by eluting with water/acetonitrile (90:10% v/v) at a flow rate of 2 mL/min and passed through the photodiode array detector (PDA-100, Dionex) set at 275 nm. Authenticity of detected peaks was determined by comparison of retention time, spectral extraction at 275 nm and spiking the sample with standard deltamethrin. A calibration curve of deltamethrin was generated by

Chromleon software (Dionex) using known amounts of the standard (0.0–0.4 µg/mL) in acetonitrile, then used to estimate the amount of deltamethrin in the net samples (four replicates for each treatment type and wash number).

Statistical analyses

In the first Tanzanian trial, nets were bioassayed after each wash (three nets), or every fourth wash (eight nets). Linear regressions of mortality and MTKD on the number of washes were calculated for each type of net, i.e. white or green PermaNet or net conventionally treated with alphacypermethrin. If significant, the regression coefficients were used to predict the mortality or MTKD values after 20 washes. In the other five trials, bioassays were done after a variety of wash cycles. Proportional data on mosquitoes (i.e. proportions dead and blood-fed) collected from the huts and platforms, and the results of 3-min exposure bioassays were analysed using blocked logistic regression (STATA 6 software). Comparisons between treatments were made by successively dropping treatments from the overall comparison. This process allowed each treatment to be compared with every other. Means and confidence limits of the constant for each treatment were back-transformed for presentation, as follows:

$$x' = \frac{1}{1 + [1/\exp(x)]}$$

where: x' = back-transformed value and x = the value from the logistic regression.

Total numbers of mosquitoes caught in the experimental huts, each night for each treatment, were compared using the Scheffe multiple comparison test to investigate any differences in repellency between the treatments. In the Pakistani trials, where enclosed platforms were used, repellency could not be measured as mosquitoes were introduced into an enclosed area from which they could not escape.

Results

First version of PermaNet

Phase I bioassays: 24-h mortality after 3-min exposure to net swatches. Anopheles mortality after contact with unwashed

Table 2. Phase I results of the First Tanzania trial: *Anopheles gambiae* median time to knockdown (MTKD) and percentage mortality 24 h after 3-min exposure, comparing the first version of PermaNet vs. nets conventionally treated with alphacypermethrin 20 mg ai/m². MTKD values are the mean of two to five tests (indicated by superscript prefix); mortality values are the mean of three replicate tests (each with 11 mosquitoes).

Type of net and treatment	Date of test (month/year)	Total no. washes ^a	3-min exposure		Median time to knockdown (s)			
			Individual nets		Nets grouped for analysis		Individual nets	
			Mortality before washing	Mortality after last wash ^c	Regression coefficient (mortality/no. washes) ^b	Predicted mortality after 20 washes	MTKD before washing (s)	MTKD after last wash (s)
PermaNet (green)	July 2000	¹ 21	100%	100.0%			³ 396	⁵ 1119
	July 2000	¹ 21 ^d	100%	87.9%	−0.40***	93.4%	³ 401	³ 1424
	August 2000	¹ 20 ^c	100%	90.9%			⁴ 426	⁴ 909
PermaNet (white)	March 2000	¹ 20 ^c	100%	100.0%			² 407	² 538
	April 2000	³ 9	100%	97.7%	[−0.55]	[100%]	² 490	⁴ 1007
	June 2000	³ 15	100%	100.0%			⁴ 514	⁴ 1422
Alphacypermethrin 20 mg ai/m ²	August 2000	¹ 21	100%	100.0%			⁴ 494	² 772
	March 2001	¹ 12	100%	40.9%			² 372	² 764
	April 2001	¹ 20 ^c	100%	86.4%	−0.65**	86.9%	² 362	² 1268
	May 2001	³ 8	100%	88.6%			² 317	⁴ 786
	June 2001	³ 14	100%	95.5%			⁴ 449	⁴ 1247

^aNumber of days between each wash and each test (1 or 3) indicated by prefix superscript.
^bRegression coefficient significance of departure from zero indicated by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001, or [not significant].
^cTested after every four washes; all other nets tested after each wash.
^dLocal soap used for washing; all other nets washed with Foma detergent.
^eConsiderable fluctuations occurred between successive bioassay test results for each net: for some nets the final bioassay mortality rate was more than some preceding values.

treated nets (the original version of PermaNet and two types of CTDN) was consistently 100% in the Iran trial and all Tanzanian studies (Tables 2 and 3). However, in the first Pakistan trial, baseline mortality was much lower after contact with unwashed treated nets, but was significantly more for PermaNet (94.2%) than for CTDNs (85.2% for deltamethrin SC 50 mg ai/m² and 69.1% for alphacypermethrin SC 15 mg ai/m²) (Table 3).

After repeated washing (the number of wash cycles ranged from 8 to 21 in different trials), bioassays on white

PermaNets gave 97.7–100% mortality (Table 3) showing no significant loss of insecticidal efficacy, whereas washed CTDNs gave significantly lower mortality rates: 70% in Pakistan (21 washes of net with 50 mg deltamethrin/m² from K-Othrin), 64.8% in Iran and 81.8% in Tanzania second trial (respectively 15 and 12 washes of nets with 25 mg deltamethrin/m² from KO-tab). Nets conventionally treated with alphacypermethrin also showed significantly reduced efficacy after washing: mortality ranged from 40.9 to 95.5% on nets treated with 20 mg ai/m² after 8–21 washes

Table 3. Phase I trials in Pakistan, Tanzania and Iran of PermaNet and three types of conventionally treated nets showing the results of 3-min exposure tests against *Anopheles* mosquitoes. Means sharing a superscript letter in the same column do not differ significantly by blocked logistic regression (*P* > 0.05).

Type of net and treatment		First Pakistan trial: 21 washes (insectary-reared, non-bloodfed <i>An. stephensi</i>)		Second Tanzania trial: 15 washes (wild-caught, blood-fed <i>An. gambiae</i>)		Iran trial: 15 washes (wild-caught, blood-fed <i>An. stephensi</i>)	
		No. tests	% mortality	No. tests	% mortality	No. tests	% mortality
PermaNet	Unwashed	8	94.2 ^a	8	100.0 ^a	5	100.0 ^a
	Washed	5	98.2 ^a	8	100.0 ^a	10	97.7 ^b
Deltamethrin SC 50 mg ai/m ²	Unwashed	8	85.2 ^b	–	–	–	–
	Washed	5	70.0 ^c	–	–	–	–
KO-tab net	Unwashed	–	–	8	100.0 ^a	5	100.0 ^a
	Washed	–	–	8	81.8 ^b	10	64.8 ^c
Alphacypermethrin SC 15 mg ai/m ²	Unwashed	8	69.1 ^c	–	–	–	–
	Washed	5	49.1 ^d	–	–	–	–
Untreated net		8	0 ^e	–	–	5	0 ^d

in Tanzania (Table 2) and 49.1% on nets treated with 15 mg ai/m² after 21 washes in Pakistan (Table 3).

The loss of insecticidal efficacy after washing of the green PermaNets tested in the first Tanzanian trial was no different from that on alphacypermethrin-treated nets (Table 2); results between individual nets were, however, not consistent.

MTKD bioassays. The median time to knockdown (MTKD) on unwashed PermaNet was around 500 s in all four trials and was similar to that on unwashed CTDNs in the first Pakistan and Iran trials (Table 4). In the latter study, the MTKD after 15 washes increased 1.7-fold on CTDN ($P < 0.001$) but did not change significantly on PermaNet ($P = 0.42$). In the second Tanzania trial (Table 4), the MTKD after 12 washes rose 2.5-fold on CTDN ($P < 0.001$) but, as in the Iranian study, showed no significant increase on PermaNet ($P = 0.262$).

By contrast, in the first Pakistan study the MTKD after 21 washes doubled on both PermaNet ($P < 0.001$) and CTDN ($P < 0.001$) (Table 4). In the third Tanzania study, the MTKD after 15 washes almost doubled on PermaNet ($P < 0.001$) and rose 2.3-fold for nets treated conventionally with alphacypermethrin 20 mg/m² (Table 4). Moreover, in the first Tanzania trial (Table 2) the mean MTKD doubled for white PermaNets after 9–20 washes ($P < 0.001$) and almost tripled for green PermaNets washed 20–21 times ($P = 0.002$), compared with increases of 1.6–3.5-fold for conventionally treated alphacypermethrin nets washed 8–21 times ($P < 0.001$).

Thus by the MTKD criterion, the first version of PermaNet showed inconsistent wash-resistance, and outperforming the comparison ITNs in three of the four phase I trials.

Phase II trials with whole nets in experimental huts and on enclosed platforms. Anopheline mosquito mortality rates were far less during the enclosed platform trials in Pakistan (Table 5) than in the experimental huts in Tanzania (Table 6). Conversely, wild culicine mortality was far less

in Tanzanian huts than in the Pakistan test setting (cf. Tables 5 and 6). In Pakistan, culicine mortality was similar to anopheline mortality rates (Table 5). Low culicine mortality has consistently been observed at this Tanzanian study site (Curtis et al., 1996) and is probably due to pyrethroid resistance (Khairandish & Wood, 1993).

Predominant species involved were *Anopheles gambiae*, *An. funestus* and *Culex quinquefasciatus* in Tanzania (Curtis et al., 1996); *An. stephensi*, *An. subpictus*, *An. nigerrimus*, *An. pulcherrimus*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. bitaeniorhynchus* and *Cx. vishnui* in Pakistan (Graham et al., 2002a, b).

The first trial in Pakistan evaluated bednets on enclosed platforms for 3 months (Table 5), comparing the first version of PermaNet vs. untreated polyester net and a net conventionally treated with deltamethrin at 50 mg ai/m² (CTDN). Figure 1 shows the insecticidal efficacy of these nets after 0, 5, 10 and 15 washes. For both types of treated net, the 24-h mortality rate rose slightly at five washes and then declined at 10 and 15 washes to below the unwashed starting level, this trend being shown also by mortality rates on the untreated (control) net, reflecting seasonal fluctuations in ambient conditions, with temperature and humidity being higher during the tests at five washes (August) than at 10 washes (September) or 15 washes (October). After correction by Abbott's formula to adjust for control mortality, the performance after washing declined less than indicated by the unadjusted data, with no significant difference between the PermaNet and CTDN. After 15 washes, the treatment-induced mortality of *An. stephensi* was reduced from 35% to 20% for the washed CTDN and from 36% to 15% for the washed PermaNet, whereas an unwashed PermaNet tested in parallel gave only 20% mortality at the end-point (Fig. 1). For both types of treated net in the first Pakistan trial, culicine mortality rates of ~16–18% did not decline significantly with washing (Table 5).

Table 4. Phase I trials in Pakistan, Tanzania and Iran of PermaNet and three types of conventionally treated nets showing the results of median time to knockdown tests (MTKD in seconds) using *Anopheles* mosquitoes. Within columns, means sharing a superscript letter do not differ significantly ($P < 0.05$).

		First Pakistan trial: 21 washes (insectary-reared, non-bloodfed <i>An. stephensi</i>)		Second Tanzania trial: 12 washes (wild-caught, blood-fed <i>An. gambiae</i>)		Third Tanzania trial: 15 washes (wild-caught, blood-fed <i>An. funestus</i>)		Iran trial: 15 washes (wild-caught, blood-fed <i>An. stephensi</i>)	
Type of net and treatment		No. tests	Mean MTKD	No. tests	Mean MTKD	No. tests	Mean MTKD	No. tests	Mean MTKD
PermaNet	Unwashed	8	549 (401–690) ^a	8	572 (482–662) ^a	22	434 (403–464) ^a	5	444 (250–704) ^a
	Washed	5	1045 (934–1155) ^b	8	633 (547–720) ^a	20	822 (719–925) ^b	5	526 (417–656) ^a
Deltamethrin SC 50 mg ai/m ²	Unwashed	8	456 (337–575) ^a	–	–	–	–	–	–
	Washed	5	1019 (879–1158) ^b	–	–	–	–	–	–
KO-tab net	Unwashed	–	–	8	396 (352–439) ^b	–	–	10	499 (273–808) ^a
	Washed	–	–	8	983 (765–1200) ^c	–	–	10	858 (656–1048) ^b
Alphacypermethrin SC 15 or 20 mg ai/m ²	Unwashed	8	523 (430–615) ^a	–	–	14	420 (374–465) ^a	–	–
	Washed	5	999 (872–1127) ^b	–	–	10	964 (727–1201) ^b	–	–
Untreated net		8	no knockdown	8	no knockdown	–	–	–	–

Target dosages of alphacypermethrin were 15 mg ai/m² in the Pakistan trial and 20 mg ai/m² in the third Tanzania trial.

Table 5. First Pakistan trial with nets on enclosed platforms, comparing the first version of PermaNet, conventionally treated deltamethrin nets (CTDN) (deltamethrin SC 50 mg/m²) and untreated nets, showing mosquito data for *Anopheles stephensi* females and *Culex* spp. females.

Type of net and treatment		No. of nights	Mean no. of mosquitoes per hut per night	% mortality
Anophelines				
PermaNet	Unwashed	15	15.7	21.2 ^a
	Washed 15 ×	15	15.4	16.5 ^a
CTDN	Washed 15 ×	15	13.4	21.5 ^a
Untreated Net		15	16.0	7.1 ^b
Culicines				
PermaNet	Unwashed	15	375.2	16.2 ^b
	Washed 15 ×	15	390.3	17.7 ^a
CTDN	Washed 15 ×	15	382.6	17.5 ^{ab}
Untreated net		15	376.4	7.2 ^c

Values sharing a superscript letter, within each column and subfamily of mosquitoes, do not differ significantly ($P > 0.05$) by blocked logistic regression analysis for percentage mortality, nor by Scheffe multiple comparison test for mean numbers caught (cf. Figure 1). Numbers of mosquitoes/night are not indicative of relative repellency/attractiveness of the net on each platform, as laboratory-reared mosquitoes were manually introduced to the enclosed nets and were unable to leave the platform.

In the second Tanzanian trial (Table 6) comparing PermaNet (first version) with CTDN (KO-tab 25 mg ai/m²), *Anopheles* mortality on PermaNet remained high (> 90%) after 15 washes, but appeared to fall on CTDN, from 84% to 70% after 15 washes, although this decline was not significant ($P = 0.162$). Culicine mortality rates were far lower on treated nets (3.3–6.7%) and not significantly different between net types, irrespective of washing. Both types of treated net stopped the majority of mosquitoes from biting successfully. Blood-feeding rates of anophelines showed no change after 15 washes of the nets, whereas culicine blood-feeding rates appeared to increase (Table 6), although this trend was not significant.

Similarly in the third Tanzanian trial, 24-h mortality rates of anophelines (86–94%) and culicines (0.4–1.2%)

were apparently unaffected after 15 washes of PermaNet (first version) and CTDN (KO-tab), both of which prevented the majority of mosquitoes from blood-feeding (Table 6).

PermaNet 2.0

Bioassays with 3-min exposure. Before washing, there were no significant differences in mortality or knockdown between PermaNet 2.0 and CTDN (Table 7). After five or 10 washes of PermaNet 2.0, there was no significant reduction of efficacy in mortality compared to unwashed PermaNet 2.0 ($P = 0.2$). After 20 washes of PermaNet 2.0, bioassays

Table 6. Second and third Tanzania trials in experimental huts comparing the first version of PermaNet and KO-tab-treated nets (CTDN: conventionally treated deltamethrin nets) before and after washing. Anophelines were nearly all *An. gambiae*, plus a few *An. funestus* in trial 3.

		Second Tanzania trial				Third Tanzania trial			
Type of net and treatment		No. of nights	Mean no. of mosquitoes per hut per night	% blood-fed	% mortality	No. of nights	Mean no. of mosquitoes per hut per night	% blood-fed	% mortality
Anophelines									
PermaNet	Unwashed	15	6.2 ^a	7.0 ^a	97.7 ^b	14	5.4 ^a	6.6 ^a	85.5 ^a
	Washed 15 ×	13	6.4 ^a	3.6 ^a	91.6 ^b	21	5.3 ^a	1.8 ^a	93.8 ^a
KO-tab net	Unwashed	13	2.4 ^a	9.7 ^a	83.9 ^{ab}	—	—	—	—
	Washed 15 ×	14	4.1 ^a	8.8 ^a	70.2 ^a	21	4.0 ^a	4.8 ^a	88.0 ^a
Untreated net		13	6.0 ^a	32.1 ^b	25.6 ^c	16	3.4 ^a	56.4	34.6 ^b
Culicines									
PermaNet	Unwashed	15	9.7 ^a	2.1 ^c	3.5 ^a	14	36.6 ^a	4.3 ^a	1.2 ^a
	Washed 15 ×	13	13.8 ^a	5.0 ^{abc}	6.7 ^a	21	34.6 ^a	5.9 ^a	0.4 ^a
KO-tab net	Unwashed	13	9.1 ^a	5.1 ^{abc}	5.1 ^a	—	—	—	—
	Washed 15 ×	14	10.8 ^a	10.6 ^b	3.3 ^a	21	27.5 ^a	3.8 ^a	0.5 ^a
Untreated net		13	7.3 ^a	9.5 ^{ab}	0 ^b	16	44.1 ^a	31.3	0 ^b

Values sharing a superscript letter, within each column and mosquito subfamily, do not differ significantly ($P > 0.05$) by blocked logistic regression analysis for percentage blood-fed, nor by Scheffe multiple comparison test for mean number caught.

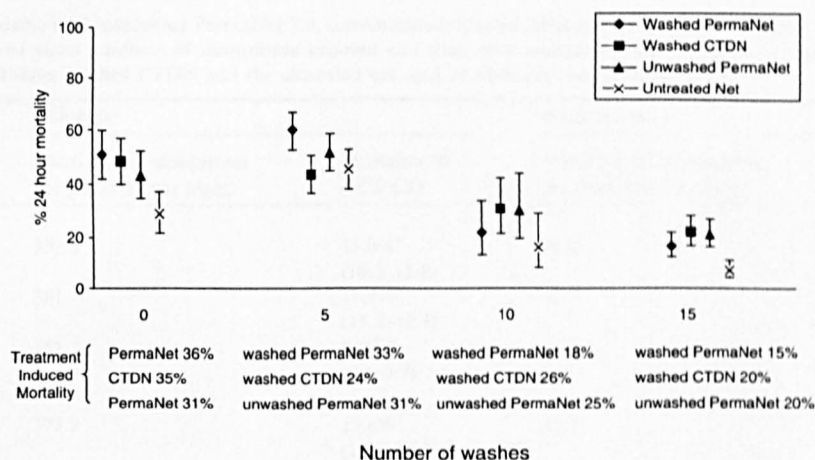


Fig. 1. First Pakistan trial, phase II results: *Anopheles stephensis* percentage mortality 24 h after starting overnight platform tests, comparing the first version of PermaNet vs. conventionally treated deltamethrin nets (CTDN: deltamethrin SC 50 mg ai/m²) washed 0, 5, 10 or 15 times. Treatment-induced mortality was calculated using Abbott's formula, corrected against the mean value for the untreated net. Data points for each number of washes are the mean of 15 successive nights (replicates): each type of unwashed net was first tested on five platforms in rotation for 15 nights; after washing up to five times they were then tested again in rotation on five platforms for 15 nights; after washing up to 10 times they were then tested again in rotation on five platforms for 15 nights; finally after washing up to 15 times the were again tested in rotation on five platforms for 15 nights.

still gave approx. 80% knockdown and mortality, although both of these were significantly lower than an unwashed PermaNet 2.0 (knockdown $P < 0.001$; mortality $P = 0.039$). After 30 washes of PermaNet 2.0 both knockdown and mortality had declined to suboptimal levels. In contrast to the swatches of LLIN, swatches of CTDN showed a dramatic reduction in efficacy after 10 water bath washes, knockdown and mortality both being very low ($P < 0.01$ for both), whilst a small proportion of mosquitoes were knocked down 1 h after exposure to the 20 or 30 times washed CTDN, these mosquitoes recovered during the 24-h holding period, resulting in no mortality.

Enclosed platform trial. Mosquito mortality rates were generally low during the second Pakistan trial and showed little variation: anophelines 14.1–21.6%, culicines 7.8–16.5% (Table 8). On the CTDN (50 mg ai/m²), mortality was significantly lower after exposure to treated nets washed 20 times than on the unwashed nets, for culicines ($P < 0.001$) or anophelines ($P = 0.032$), but not when washed 10 times only. By contrast, there was no decline in mortality on PermaNet 2.0 after 20 washes, for culicines

($P = 0.636$) or anophelines ($P = 0.993$). With the untreated net, the proportion of culicines (4.3%) and anophelines (9.0%) blood-feeding was too low for detection of any treatment-induced effects.

Chemical assay of deltamethrin concentration on nets (second Pakistan trial). Deltamethrin content on unwashed whole PermaNet 2.0 was found to be very variable (27–142 mg/m²), although the mean value of 55.3 (95% CI: 10.1–100.6) mg/m² came very close to the target concentration. Generally, the HPLC assay data (Table 9) showed low variability in deltamethrin content between samples at each wash point.

Swatches of PermaNet 2.0 had a high deltamethrin content before washing (mean 86.3, range 83–92 mg/m²), which fell by almost half over the first 10 washes, by another ~50% over the next 10 washes, and by 75% between 20 and 30 washes. On average, the first 10 washes removed 37 mg/m² and the final 10 washes removed 18 mg/m² of insecticide. A similar rate of removal was observed for machine-washed PermaNet 2.0; deltamethrin content fell by ~50% after 10 washes and by a further 50% after 20 washes.

Table 7. Second Pakistan trial comparing swatches of PermaNet 2.0 and conventionally treated deltamethrin nets (CTDN: 50 mg ai/m²), washed and unwashed and tested in LSHTM with *Anopheles stephensi* Beech using 3-min exposure: mean percentage knockdown 1-h post-exposure; mean percentage mortality 24-h post-exposure (95% confidence interval, CI). Percentage mortality, knockdown and CIs are backtransformed from values calculated by the blocked logistic regression model. Negative control mortality (tests on untreated net) were always < 5%. Four replicates for each point.

	Mortality percentage (95% CI)				Knockdown percentage (95% CI)			
	0 washes	10 washes	20 washes	30 washes	0 washes	10 washes	20 washes	30 washes
PermaNet 2.0	97.7 (85.3–99.7)	90.7 (77.7–96.5)	81.8 (67.7–90.6)	43.2 (29.5–58.0)	100 (100–100)	97.7 (85.3–99.7)	79.5 (65.1–89.0)	77.3 (62.7–87.3)
CTDN	90.9 (78.2–96.5)	14.0 (6.4–27.8)	0 (0)	0 (0)	93.2 (80.9–97.8)	46.5 (32.3–61.3)	11.4 (4.8–24.5)	2.3 (0.3–14.4)

Table 8. Second Pakistan trial comparing PermaNet 2.0, conventionally treated deltamethrin nets (CTDN), and untreated net on enclosed platforms, showing the mean numbers of mosquitoes exposed and their 24-h mortality rates. Numbers of replicate nights were 20 for unwashed CTDN, 10 times washed CTDN and the untreated net, and 21 nights for all other net types.

	Culicines		Anophelines	
	Mean no. of mosquitoes per platform per night	Mortality % (95% CI)	Mean no. of mosquitoes per platform per night	Mortality % (95% CI)
CTDN				
Unwashed	332.6	11.0% ^a (10.3–11.8)	35.6	18.3% ^{ab} (15.6–21.3)
Washed 10 ×	361	11.9% ^a (11.2–12.7)	38	21.1% ^a (18.3–24.1)
Washed 20 ×	352.7	9.0% ^b (8.4–9.7)	32.9	14.1% ^b (11.7–16.9)
PermaNet 2.0				
Unwashed	373.2	13.6% ^c (12.9–14.4)	37.8	20.6% ^a (17.9–23.5)
Washed 10 ×	364.2	16.5% ^d (15.7–17.3)	30.6	21.6% ^a (18.6–25.0)
Washed 20 ×	368.6	13.9% ^c (13.1–14.7)	30.1	20.5% ^a (17.6–23.9)
Untreated net	402.8	7.8% ^e (7.2–8.4)	36.3	16.1% ^b (13.6–19.0)

Within columns, values not sharing a superscript letter differ significantly ($P < 0.05$).

Numbers of mosquitoes per night do not provide information about the repellency or attractiveness of treatments, as wild-caught mosquitoes were introduced manually and were unable to leave the trap nets over the platforms.

Deltamethrin concentrations remaining on PermaNet 2.0 after 20 washes in the water bath (24.1 mg/m²) or washing machine (13.1 mg/m²) were similar to the range normally recommended for conventionally treated nets (W.H.O., 1999), before washing. However, this impressive amount withstanding 20 washes appears to have been at least partly due to the high loading dose of deltamethrin at the point of manufacture.

Before washing, the insecticide dosage was found to be higher on all the treated net swatches destined for water bath washing than on the equivalent whole nets destined for machine washing (Table 9). This may have been the result of

whole nets being handled far more than the net swatches. As the whole nets (unwashed and washed) had been field-tested on enclosed platforms before HPLC assay, they might have lost some insecticide by weathering and abrasion during use.

These two methods of washing readily stripped deltamethrin from the CTDNs, so that very low amounts were detectable after 10–20 washes (anomalous detection of 29 mg/m² on one piece after 20 washes may indicate uneven stripping or contamination). On 16 swatches of CTDN, with one exception, no deltamethrin was detected after washing 10 or 20 times.

Table 9. High-performance liquid chromatography determinations of deltamethrin concentrations on nets evaluated in the second Pakistan trial.

	Mean deltamethrin content mg/m ² (95% CI) [range]			
	0 washes	10 washes	20 washes	30 washes
PermaNet 2.0 (net swatches washed in waterbath)	86.3 (83.9–88.7) [83.1–91.7]	49.5 (47.4–51.5) [46.0–53.0]	24.1 (20.7–27.4) [16.4–29.8]	5.8 (4.3–7.4) [2.8–8.1]
CTDN (net swatches washed in waterbath)	22.9 (19.9–25.8) [18.0–27.5]	not detected	3.6* (–4.9–12.1) [0–28.8]	tests not done
PermaNet 2.0 (whole net washed in machine)	55.3 (10.1–100.6) [27.1–142.0]	22.8 (18.0–27.6) [20.0–26.0]	13.1 (11.2–14.9) [10.8–15.2]	tests not done
CTDN (whole net washed in machine)	11.9 (9.0–14.9) [8.0–15.2]	1.5 (0.9–2.0) [0.8–2.0]	1.4** (1.0–1.8) [0–1.6]	tests not done

*Detected in only 1 of 8 samples.

**Not detected in 2 of 6 samples.

The phase I bioassays on these net swatches suggest that levels of insecticide too low to be detected by HPLC may still cause temporary knockdown though not mortality: mosquitoes knocked down 1 h after exposure to the 10 and 20 times washed swatches of CTDN recovered during the 24-h holding period.

Discussion

Our five trials of the first version of PermaNet gave inconsistent results on its wash-resistance compared to conventionally treated nets. Trials by other investigators (Gonzalez *et al.*, 2002; Müller *et al.*, 2002; W.H.O., 2004a) also reported inconsistency of this product. Even so, from bioassay mortality tests (with 3-min exposure) on net swatches we concluded that the original PermaNet performed better than conventionally treated nets after multiple washes (ranging from 8 to 21 washes) in all but the first Tanzanian trial. Although the efficacy of conventional ITNs (treated with alphacypermethrin or deltamethrin) did decline more with repeated washing, these treatments were by no means exhausted, even after 21 washes. This finding conflicts with the widely held view that, after conventional pyrethroid impregnation by dipping (Chavasse *et al.*, 1999), ITNs lose insecticidal activity after only a few washes. Partial wash-fastness is an inherent property of alpha-cyano-pyrethroids on polyester (N'Guessan *et al.*, 2001).

Median time to knockdown (MTKD) bioassays have the capacity to reveal small differences in performance when the amount of bioavailable insecticide remains sufficient to produce uniformly high mortality in 3-min bioassays. The evidence from MTKD for superior wash-resistance of the earlier PermaNet was equivocal: the Iran and second Tanzania trials showed slight but non-significant increase of the MTKD after washing, whereas in three other trials (Pakistan and Tanzania first and third) the insecticidal efficacy of PermaNet declined like that of conventionally treated nets, the MTKD approximately doubling after washing across all three studies.

These inconsistencies within and between our five independent studies of the first version of PermaNet suggest that this promising LLIN technology was being adversely affected by variation in quality within or between batches of nets. Contradictions between findings of Gonzalez *et al.* (2002) and Müller *et al.* (2002) in their tests of the earlier PermaNet might also be explained by batch variation. The manufacturer has accepted this problem of variability in the production process (Kilian, 2004), and since this discovery the manufacturer has improved quality control and relaunched the LLIN as 'PermaNet 2.0' in late 2002.

Using the interim WHO standard water bath protocol (section 3.3.1 in WHO, 2004a) in our Phase I tests on PermaNet 2.0, we showed a far superior retention of efficacy after washing of the LLIN compared to the conventionally treated net. The chemical analysis of deltamethrin content gives further evidence for a superior wash resistance of PermaNet 2.0, which demonstrated high insecticide load

at 20 washes and still detectable insecticide at 30 washes. By contrast, on the CTDN, deltamethrin levels on net swatches were too low to be detected after 10 washes.

Before undertaking these trials we hypothesized that the superior performance of PermaNet 2.0 might be due to the high loading dose of deltamethrin in the LLIN (confirmed by HPLC analysis here) rather than to improved wash resistance. Further investigation has gone some way to refuting this: a phase I comparison of PermaNet 2.0 with a net treated conventionally with two KO-tabs (deltamethrin ~50 mg ai/m²) after washing with the water bath procedure, demonstrated that the high loading dose was not the sole cause of the superior performance (Kayedi, 2004).

The advantage of phase I bioassays of residual activity, such as the MTKD and 3-min exposure tests, for comparative evaluation of ITNs and LLINs, lies in their ability to reveal small differences in performance between treatments. Although this is useful for assessing incremental improvements in LLIN technology, it is not known what magnitude of difference in such bioassays would translate to an effect that would be detectable at the levels of vector control or disease impact. Before an informed decision can be taken as to whether a new technology offers sufficient improvement over an existing technology to justify the costs and effort of substitution, more information than that given by simple residual bioassays is needed. Field trials (phase II) with free-flying mosquitoes, enabling observations of more natural interactions between females and the insecticidal net, provide a better indication of LLIN performance in everyday use.

Improved bioassay mortality rates achieved with some early batches of PermaNet did not necessarily translate to a detectable reduction of blood-feeding success or survival rates in field comparisons with conventionally treated nets. For example, in the Pakistan enclosed platform trial and in one of the two trials in Tanzanian huts, performance of the earlier PermaNet remained equivalent, not superior, to that of conventionally treated nets, even after 15 washes. Only in the second Tanzania trial did PermaNet show superior performance to CTDN at the point of 15 washes. This illustrates that the earlier batches of PermaNet were of variable quality, an inference recently acknowledged in the 7th WHOPES Working Group Report (W.H.O., 2004a).

On the basis of these results and two other field trials (Akogbeto *et al.*, 2003; Kilian, 2004) and one other phase I trial (Duchon *et al.*, 2003), PermaNet 2.0 obtained WHOPES interim recommendation for use in prevention and control of malaria (section 3.5 in W.H.O., 2004a). It is important that evaluation of PermaNet 2.0 in everyday use takes place in different cultural and epidemiological settings, both to examine the effectiveness of this LLIN in various settings and to ensure that the variable quality hampering the usefulness of the first version of PermaNet has been resolved.

With LLIN technology now being embraced by control programmes worldwide, there is likely to be a proliferation of manufacturers bringing such products to the market. Several LLIN products are currently under development.

Draft LLIN specifications have been prepared (F.A.O./W.H.O. 2004), but methods for evaluating LLINs need standardization to distinguish LLINs from less durable or even fraudulent LLINs, bearing in mind that most ITNs retain at least some insecticidal power after washing. Gonzalez *et al.* (2002) found that soaking and more vigorous washing was needed to differentiate between CTDNs and PermaNet. Here we show that the rigorous water bath wash protocol, adopted *ad interim* for WHOPES purposes (section 3.3.1 in WHO, 2004a), demonstrated a clear difference between the LLIN and CTDN. For proving the superiority of LLINs over conventionally treated nets in everyday use, or to demonstrate incremental improvements in performance of LLINs, a standardized wash protocol that equates to the most vigorous wash likely to be used in everyday use is required. There is still no consensus on what should constitute a standardized wash for whole nets. Current wash protocols do not incorporate a realistic time interval between washes, whereas in everyday use, owners may wash nets infrequently or irregularly at intervals of weeks, months or even years. In accelerated trials, it is convenient to re-wash rapidly, even daily, which may be insufficient interval to allow reactivation of certain types of LLIN such as Olyset Net[®] (W.H.O., 2001, 2004b), which has insecticide incorporated within the fabric and requires more time to restore potency by diffusion of active ingredient from within the fibre to the surface. However, the practicalities of washing LLINs more than 20 times in preparation for testing means that intervals between successive washes should be no more than a week if studies are to be done within a reasonable time frame.

LLIN technology is not just about insecticide wash-resistance but also provision of long-term effectiveness against mosquitoes during regular use. Important points bearing on effectiveness include user acceptability, the physical lifespan of the net and treatment, migration of insecticide to the surface and sustained availability during long-term use, interaction with mosquito tarsi and uptake when they stand on the net. Beyond experimental hut and platform trials, another critical LLIN requirement is the retention of sufficient insecticide on nets after several years of use by householders. The PermaNets bioassayed after 20 washes by Gonzalez *et al.* (2002) were then given to Colombian villagers for everyday use. When the deltamethrin concentration was assayed after 3 years of domestic usage, with regular washing, the residue had dropped from the original 55 mg/m² to an average of 9.6 mg/m² (Kroeger *et al.*, 2004). Even so, bioassay mortality remained at 88%. Unfortunately no CTDNs with similar usage history were available for controlled comparison. Elsewhere, user studies conducted in Malawi and Uganda did compare the earlier PermaNet against CTDN controls, and tests showed marked declines in efficacy and deltamethrin content within 6–24 months of use, less for PermaNet than for CTDN (W.H.O., 2004a). Results of phase I, phase II and chemical assays of PermaNet 2.0 are impressive so far, but the *interim recommendation* conferred by WHOPES (W.H.O., 2004a) reflects the need for evidence of long-lasting efficacy over several years of use in different settings.

As PermaNet 2.0 is poised to supply the demands of institutional donors and malaria control programmes, it must be remembered that many millions of bednets in routine household use are conventionally treated ITNs or untreated nets. The need for regular re-treatment of conventional ITNs should not be neglected and strategies to maintain the effectiveness of ITNs, while establishing the reliability of LLINs, will remain vital for years to come.

Acknowledgements

Trials in Pakistan were conducted under the auspices of HealthNet International (HNI, <http://www.healthnetinternational.org>). HNI's Malaria Control Programme in Pakistan was supported by the European Commission and PermaNet studies supported by the World Health Organization Pesticide Evaluation Scheme (<http://www.who.int/whopes>) with advice from Morteza Zaim and Pierre Guillet at W.H.O. Headquarters. The Bed Net Research Programme in Tanzania was supported by the British Medical Research Council. We wish to thank Ali-A kbar Haghdoust in Iran (and at LSHTM), Joseph Myamba in Tanzania, Mushtaq Ahmad, Kamal Mohammed, Irfanullah Khan, Abdullah Nazari and Chris White in Pakistan for field operations. M.W.R. and H.K. are supported by the Gates Malaria Partnership, J.D.L. by the Department for International Development, C.F.C. and C.M. by the Medical Research Council, and M.K. by the Iranian government. None of these donors can accept responsibility for any information provided or views expressed.

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Accepted 27 November 2004

no longer be guaranteed. A few brief case examples illustrate the difficulties facing health care delivery under these new conditions.

BALKANS - BOSNIA/SARAJEVO 1992-1995

While now recovering, Bosnia-Herzegovina in the 1990s fitted well the definition of a failed state. Sarajevo was subjected to the longest siege in modern times, lasting from the spring of 1992 to the autumn of 1995. Expatriate health professionals reported the consequences describing the deliberate targeting of medical infrastructure and personnel during the siege of the city.^{2,3} With an increasing influx of wounded into the hospital system, the organisation of health care orientated towards trauma to the detriment of those with chronic medical conditions, including malignant disease. Further, hospitals with scarce fuel and power resources were unable to attend to specialist investigation and powerful technology-based forms of treatment.

Another under-reported health consequence of the Sarajevo siege was a slow and progressive starvation of the population, which was most keenly felt by vulnerable groups such as children, nursing mothers, the ill and the elderly.³ A further unsurprising side effect of severe malnutrition was noted in the city hospitals. This was a significantly increased incidence of pin track infections when reviewing patients whose fractures had been treated by external fixation.⁴

BALKANS - PRISTINA/KOSOVO 1999-2000

This region suffered a protracted civil war with large-scale forced migration. Following occupation by NATO there followed further population migration. The health consequences were catastroph-

ic. Returning refugees and internally displaced persons faced a region without a functioning government, largely destroyed housing stock and failed infrastructure. One of the most pressing needs was health care. The health problems listed below are illustrative but far from comprehensive:

- Collapse of water, power and sanitation. A particular problem was the disposing of large amounts of clinical waste in hospitals and health centres. Mortuaries were inundated with bodies lying in corridors and entrances.
- Rehabilitation of the entire health care system including primary health care, transport, communications and hospital-based systems. A particular anxiety related to the collapse of public health surveillance and reporting.
- Total systems failure at the region's 2,400 bed tertiary referral, university teaching hospital in Pristina. This was compounded by a departure of the pre-war hospital staff, mainly Serbs, and an influx of Albanian medical staff, few of whom had any proof of identity or qualification.

Such a near total failure of the instruments of government required a complete takeover of the functions of the state by the UN Interim Administration with the World Health Organization taking the health portfolio.

MIDDLE EAST - IRAQ 2003-2004

While the crisis in Iraq is on-going, it illustrates many of the problems of a failed

state. The country's health care system, once on a par with middle-income European states, had deteriorated due to the Gulf war of 1990-1991, ten years of sanctions which followed, and finally the invasion in 2003 of a US-led coalition.

The author has visited southern Iraq on numerous occasions since the invasion, concentrating on health needs assessment and health promotion. The health problems in the post-conflict period (if it can be termed post-conflict) are many and diverse. While there was no mass movement of people or forced migration there were serious and unforeseen health consequences, mainly caused by sanctions and, to a lesser extent, the recent war. The most pressing needs relate to a failure of the public health surveillance and health information systems. In short, it is still very difficult to quantify the health needs and agree priorities. Maternal and child health schemes have failed, with catastrophic maternal and infant mortalities being reported, but these are unverifiable. Until the security situation improves it is too dangerous for local or expatriate health professionals to travel to the regions and re-establish accurate health information systems - a prerequisite if health care is to be effective.

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New tools to control malaria in refugee camps

Forty per cent of the world's population live in areas where malaria transmission occurs, leading to an estimated 300-500 million cases of acute malaria per year, and

more than one million deaths.¹

Approximately 75% of countries currently undergoing complex emergencies are endemic for malaria. In such countries

malaria is one of the five main diseases contributing to the elevated mortality rates during the early stage of emergencies.² When an emergency occurs in a malaria

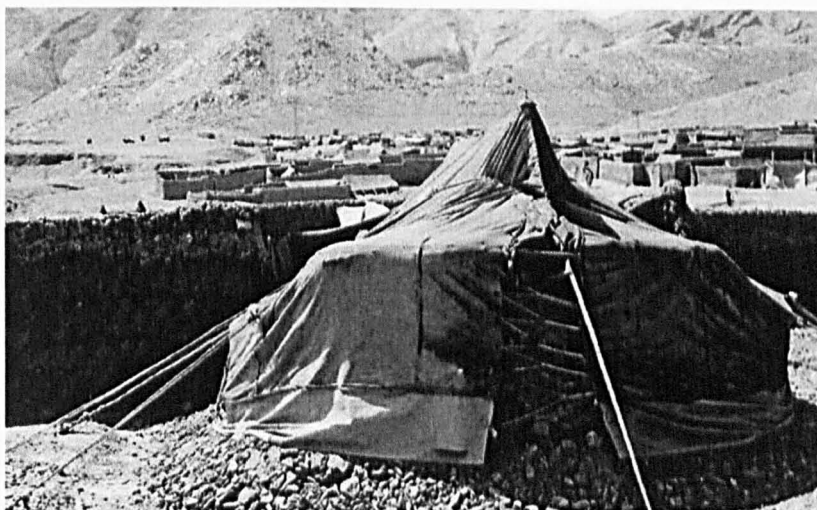
254 CURRENT TOPICS AND OPINIONS Malaria in refugee camps

endemic area there are several reasons why malaria incidence rises and the risk of epidemics increases: i) non-immune displaced people may be newly exposed to malaria transmission; ii) concentrations of people living in poor shelters facilitate transmission; iii) existing control programmes may have collapsed; and iv) treatment services may have collapsed and those in neighbouring countries may be overwhelmed by refugee influx.³⁻⁵

Delivery of insecticide-treated nets (ITNs) with concurrent health education is now the main method of malaria control advocated for stable settings. However, in a newly set up refugee or internally displaced persons (IDP) camp there are serious constraints to such an intervention. Large quantities of nets and insecticide are needed and the procurement of these, even if a stockpile is available nearby, delays intervention at the time of the highest mortality. A concurrent health education campaign is important to support maintained and consistent net use. Yet time is needed to plan this in line with the local culture, and implementation may be hindered both by the often poor co-ordination among the multitude of agencies present and by the fact that a traumatised and displaced population may not be receptive. At the early stages of an emergency, nets are therefore not the most appropriate tool.

Indoor residual spraying of houses is a malaria control tool that can achieve a rapid and large reduction in disease provided a comprehensive spray campaign can be organised. Insecticide spraying of tents has been shown to lower malaria incidence in epidemics in nomadic tribes in Pakistan⁶ and has been adopted as a method of controlling malaria in refugee camps. However, constraints similar to those impeding implementation of an ITN campaign during the early stages of an emergency are faced: trained spray-men, specialised equipment, large quantities of insecticide, and good co-ordination between agencies are all required but generally absent at this early stage.

To provide some new vector control options for this problematic early stage, when mortality is at its highest and the need for control at its greatest, new tools which are linked to the general immediate priorities during camp establishment are under evaluation. The accepted priorities



Tent modified by refugees - note the construction of internal wall

during an emergency are measles immunisation and the provision of clean water, sanitation, food, shelter and emergency health care.⁷⁻⁹ Shelters that are pre-treated with insecticide have been developed with the aim of getting a vector control tool in place as soon as the camp is established, bypassing the need for an insecticide spray campaign once a camp is already set up.

Plastic sheeting is often distributed as a shelter material when camp populations are extremely high, as it is lighter and cheaper than tents, keeping freight and purchase costs down. Plastic sheeting pre-treated (during manufacture) with deltamethrin is now in the final stages of evaluation. It has been shown to kill mosquitoes in entomological trials both in the laboratory and in the field¹⁰ remaining effective for at least six months. Disease control trials are now drawing to a close in Sierra Leone.¹¹ Pre-empting the results of these trials discussions have begun on the replacement of the untreated plastic sheeting with the pre-treated version.¹¹ Canvas tents are an alternate shelter item often distributed, providing a more robust structure and better privacy, but are considerably more expensive than plastic sheeting. A tent constructed from untreated canvas with insecticide-treated polyethylene threads woven through during manufacture has been shown to both kill mosquitoes and, as a secondary effect of the insecticide, reduce the number of mosquito bites on people sleeping within.¹² The persistence of the insecticidal effect is still under evaluation. With

evidence of the protective effect of insecticide-treated tents already in place⁶ it may be possible to use such pre-treated tents operationally without the need for new disease control trials.

With existing evidence for the efficacy of insecticide treated-tents as a malaria control tool⁶ and initial results of the efficacy of insecticide-treated plastic sheeting reported to be good¹¹ there is hope for a breakthrough in the problem of vector control for malaria at the early stages of emergencies. However, this evidence of efficacy does not ensure these tools will be effective when used operationally in the refugee camp environment, and there are probable limitations to their usefulness.

Insecticide-treated shelters protect from malaria in the same way as indoor-residual spraying: mosquitoes rest on the walls and roof of the shelter after taking a blood-meal and pick up a lethal dose of insecticide which leads to an overall reduction in the age of the mosquito population and its potential of transmitting the disease. The protective effect is dependent on a large proportion of the shelters in an area being treated and the mosquitoes resting on these treated surfaces. The plastic sheeting distributed in emergencies provides meagre shelter and living space; minimal or no materials for erection are provided with the tarpaulins. Methods of erection therefore vary considerably as inhabitants attempt variably to maximise space, privacy or airflow depending on the environment and local culture. The

entomological evaluations of the pre-treated sheeting examined structures erected in the classic ridge-pole format. The disease control trials underway are evaluating this sheeting as a partial or total lining for huts. How much protection will be provided by the numerous ways the sheeting will actually be used is unclear. The use of tents is more predictable as poles and ropes for construction are provided with the tent and there are seemingly few options for alternative erection techniques.

Both forms of temporary shelter will only provide protection for as long as they are being used in the way envisaged by the manufacturers and researchers. Refugee and IDP populations are seen to construct

more permanent shelters, or make improvements to shelters provided soon after arrival, perhaps within two to three months (see photo). Once internal walls or ceilings begin to be constructed, the insecticide in the original shelter is no longer available to resting mosquitoes. If new whole constructions are made, plastic sheeting may, for example, be relocated as waterproofing for the roof, stored for future use or sold, negating its usefulness as a vector control tool.

New pre-treated shelter tools are a promising development in the options for malaria control in refugee and IDP camps. Their usefulness will focus on the early stages of an emergency when other options are not feasible. When an emergency arises,

moves to get spray equipment and expertise in place should be initiated. Once the usefulness of the pre-treated shelters begins to be compromised, perhaps a few months into the emergency, it may then be possible to carry out a spray campaign to ensure the population remains protected regardless of the types of shelter in which they are living.

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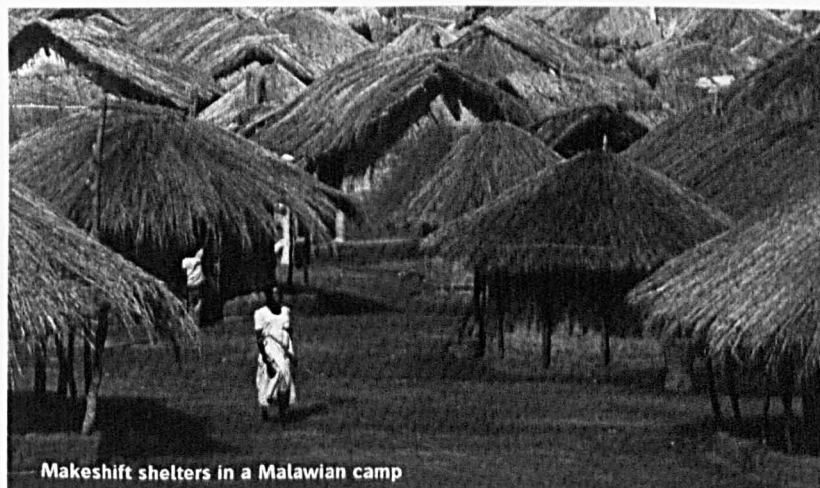
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For the want of a chicken...

It was definitely the chicken, or rather the lack of it, that was the focus of his concern. Here I was, sitting on a rush mat beside one of some five thousand makeshift shelters in the Malawian camp, conversing with a man who had lost livelihood, home and family to the civil war in Mozambique. Getting firewood involved dangerous journeys back across the border. Maize rations were very limited, and pigeon peas the only vegetable generally available. Nutritional disorders and general ill health were widespread. But it was the chicken that was currently his major worry, not that he himself would have been eating much of it. "The way it was before, if I had a visitor I could kill a chicken and give



Makeshift shelters in a Malawian camp

Photo by Agor